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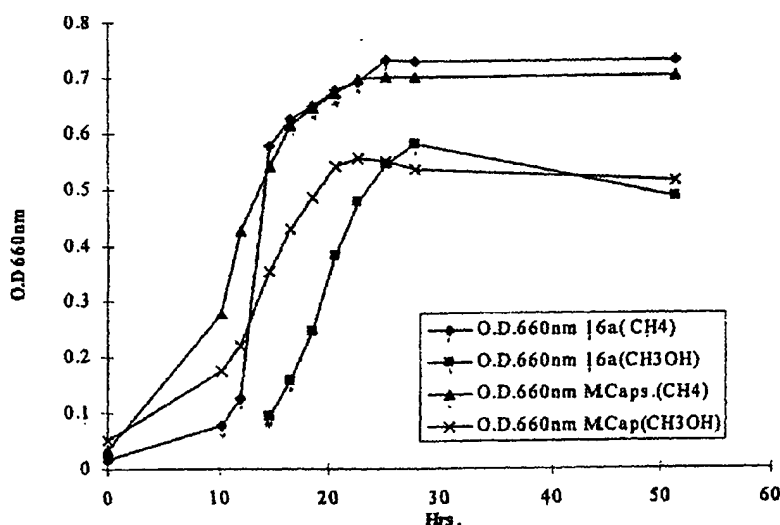
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[Continued on next page]

(54) Title: **HIGH GROWTH METHANOTROPHIC BACTERIAL STRAIN**

**16a vs. MCapsulatus Growth Curve**



(57) Abstract: A high growth methanotrophic bacterial strain capable of growth on a C1 carbon substrate has been isolated and characterized. The strain has the unique ability to utilize both methane and methanol as a sole carbon source and has been demonstrated to possess a functional Embden-Meyerhof carbon flux pathway. The possession of this pathway conveys an energetic advantage to the strain, making it particularly suitable as a production platform for the production of biomass from a C1 carbon source.



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## TITLE

### HIGH GROWTH METHANOTROPHIC BACTERIAL STRAIN

This application claims the benefit of U.S. Provisional Application No. 60/229,858 filed September 1, 2000.

5

## FIELD OF THE INVENTION

The invention relates to the field of microbiology. More specifically, the invention relates to the use of a novel methanotrophic bacterial strain capable of utilizing a central carbon pathway for more efficient production of commercially useful products.

10

## BACKGROUND OF THE INVENTION

Methanotrophic bacteria are defined by their ability to use methane as their sole source of carbon and energy. Although methanol is an obligate intermediate in the oxidation of methane, the ability to grow on methanol alone is highly variable among the obligate methanotrophs due to its toxicity (Green, Peter. *Taxonomy of Methylophilic Bacteria*. In: *Methane and Methanol Utilizers* (Biotechnology Handbooks 5) J. Colin Murrell and Howard Dalton eds. 1992 Plenum Press NY, pp. 23-84). Methane monooxygenase is the enzyme required for the primary step in methane activation and the product of this reaction is methanol (Murrell et al., *Arch. Microbiol.* (2000), 173(5-6), 325-332). This reaction occurs at ambient temperatures and pressures, whereas chemical transformation of methane to methanol requires temperatures of hundreds of degrees and high pressure (Grigoryan, E. A., *Kinet. Catal.* (1999), 40(3), 350-363; WO 2000007718; US 5750821). It is this ability to transform methane under ambient conditions along with the abundance of methane that makes the biotransformation of methane a potentially unique and valuable process.

The commercial applications of biotransformation of methane have historically fallen broadly into three categories: 1) Production of single cell protein, (Sharpe D. H. *BioProtein Manufacture* (1989). Ellis Horwood series in applied science and industrial technology. New York: Halstead Press) (Villadsen, John, *Recent Trends Chem. React. Eng.*, [Proc. Int. Chem. React. Eng. Conf.], 2nd (1987), Volume 2, 320-33. Editor(s): Kulkarni, B. D.; Mashelkar, R. A.; Sharma, M. M. Publisher: Wiley East., New Delhi, India; Naguib, M., *Proc. OAPEC Symp. Petroprotein*, [Pap.] (1980), Meeting Date 1979, 253-77 Publisher: Organ. Arab Pet. Exporting Countries, Kuwait, Kuwait); 2) epoxidation of alkenes for production of chemicals (U.S. 4,348,476); and 3) biodegradation of chlorinated pollutants

- (Tsien et al., *Gas, Oil, Coal, Environ. Biotechnol.* 2, [Pap. Int. IGT Symp. *Gas, Oil, Coal, Environ. Biotechnol.*], 2nd (1990), 83-104. Editor(s): Akin, Cavit; Smith, Jared. Publisher: Inst. Gas Technol., Chicago, IL.; WO 9,633,821; Merkle et al., *Biorem. Recalcitrant Org.*, [Pap. Int. *In Situ* On-Site Bioreclam. Symp.], 3rd (1995), 165-74. Editor(s): Hinchee, Robert E; Anderson, Daniel B.; Hoeppel, Ronald E. Publisher: Battelle Press, Columbus, OH.; Meyer et al., *Microb. Releases* (1993), 2(1), 11-22). Only epoxidation of alkenes has experienced little commercial success due to low product yields, toxicity of products and the large amount of cell mass required to generate products.

Large-scale protein production from methane, termed single cell protein or SCP has been technically feasible and commercialized at large scale (Villadsen *supra*). However, SCP has been less than economically successful due to the relatively high cost to produce microbial protein compared to agriculturally derived protein (i.e. soy protein). Single cell protein is a relatively low value product and therefore economic production cannot tolerate heavy bioprocessing costs. For this reason the yield of the methanotrophic strain may be critical to the overall economic viability of the process. Microbial biomass produced by methanotrophic bacteria is typically very high in protein content (~70-80% by weight), which can restrict the direct use of this protein to certain types of animal feed.

The conversion of C1 compounds to complex molecules with C-C bonds is a difficult and capital intensive process by traditional chemical synthetic routes. Traditionally, methane is first converted to synthesis gas (mixtures of hydrogen, carbon monoxide and carbon dioxide), which is then used to produce other small molecular weight industrial precursors. Typically these are "commodity" type chemicals such as acetate, formaldehyde, or methanol. The basic problem is activation of the methane molecule which is thermodynamically very difficult to achieve by chemical means. "Activation" refers to the process of making the chemically unreactive methane molecule more reactive.

Methanotrophic bacteria contain enzymes (methane monooxygenases) which are capable of methane activation at ambient temperatures and pressures. Methane activation consists of oxygen insertion into methane to form methanol which is much more readily metabolized to more complex molecules within the cell. Two types of methane monooxygenase are found in methanotrophic bacteria. A

particulate methane monooxygenase (pMMO) has a narrow substrate specificity and is incapable of oxygen insertion into more complex molecules. Some, but not all methanotrophs may also contain a soluble methane monooxygenase (sMMO). This enzyme has been the subject of much investigation and proprietary claims due to its ability to oxygenate, or functionalize, a wide variety of aliphatic and aromatic molecules. This characteristic has been utilized for co-metabolic production processes where methanotrophs are fed both methane and a more complex molecule to be transformed by the sMMO. Numerous examples are reported of processes requiring both methane and, typically, a petroleum-derived feedstock such as toluene, naphthalene, or decane, where sMMO plays a role. However, the art is silent with respect to using methanotrophs for net synthesis of chemicals from methane as opposed to these co-metabolic transformations. For net synthesis, only inexpensive methane is required along with the ability to genetically engineer the strain to produce the desired chemical.

Methanotrophic cells can further build the oxidation products of methane (i.e. methanol and formaldehyde) into more complex molecules such as protein, carbohydrate and lipids. For example, under certain conditions methanotrophs are known to produce exopolysaccharides (Ivanova et al., *Mikrobiologiya* (1988), 57(4), 600-5; Kilbane, John J., II *Gas, Oil, Coal, Environ. Biotechnol.* 3, [Pap. IGT's Int. Symp.], 3rd (1991), Meeting Date 1990, 207-26. Editor(s): Akin, Cavit; Smith, Jared. Publisher: IGT, Chicago, IL). Similarly, methanotrophs are known to accumulate both isoprenoid compounds and carotenoid pigments of various carbon lengths (Urakami et al., *J. Gen. Appl. Microbiol.* (1986), 32(4), 317-41). Although these compounds have been identified in methanotrophs, they have not been microbial platforms of choice for production as these organisms have very poorly developed genetic systems, thereby limiting metabolic engineering for chemicals.

A necessary prerequisite to metabolic engineering of methanotrophs is a full understanding, and optimization, of the carbon metabolism for maximum growth and/or product yield. Obligate methanotrophs are typically thought to channel carbon from methane to useful products and energy via the Entner-Doudoroff Pathway which utilizes the keto-deoxy phosphogluconate aldolase enzyme (Dijkhuizen, L., P.R. Levering, G.E. DeVries 1992. In: *Methane and Methanol Utilizers* (Biotechnology

Handbooks 5) J. Colin Murrell and Howard Dalton eds. 1992 Plenum Press NY pp 149-181). This pathway is not energy-yielding as is the case for the Embden-Meyerhof pathway. Thus, utilization of the Entner-Doudoroff pathway results in lower cellular production yields and a greater proportion of the carbon produced as carbon dioxide compared to organisms that use the Embden-Meyerhof pathway. Therefore, a more energy efficient carbon processing pathway would greatly enhance the commercial viability of a methanotrophic platform for the generation of materials.

As noted above, methanotrophic bacteria possess the potential to be commercially effective production platforms for materials such as single cell protein, exopolysaccharides, and long chain carbon molecules such as isoprenoids and carotenoid pigments. The usefulness of methanotrophs for production of a larger range of chemicals is constrained however, by several limitations including, relatively slow growth rates of methanotrophs, limited ability to tolerate methanol as an alternative substrate to methane, difficulty in genetic engineering, poor understanding of the roles of multiple carbon assimilation pathways present in methanotrophs, and potentially high costs due to the oxygen demand of fully saturated substrates such as methane. The problem to be solved therefore is to develop a fast-growing, high yielding methanotroph capable of receiving foreign genes via standard genetic procedures. Full and rapid resolution of central carbon pathways is essential for enabling pathway engineering and carbon flux management for new products.

Applicants have solved the stated problem by providing a methanotrophic bacterial strain capable of efficiently using either methanol or methane as a carbon substrate. The strain is also metabolically versatile in that it contains multiple pathways for the incorporation of carbon from formaldehyde into 3-C units. The discovery of a phosphofructokinase and fructose 1,6 bisphosphate aldolase in this strain suggests that it can utilize the more energetically favorable Embden-Meyerhof pathway in addition to the Entner-Doudoroff pathways. The present strain is shown to be useful for the production of a variety of materials beyond single cell protein to include carbohydrates, pigments, terpenoid compounds and aromatic compounds. The formation of large amounts of carbohydrates from methane or methanol can be carried out by this strain. This is surprising and also enables this strain to be used for the production of

typical carbohydrate or sugar fermentation end-products such as alcohols, acids and ketones. The present strain was also shown to be capable of genetic exchange with donor species such as *Escherichia coli* via a standard genetic procedure known as bacterial conjugation. In this way, the strain can be engineered for net synthesis from methane to produce new classes of products other than those naturally produced.

#### SUMMARY OF THE INVENTION

The present invention provides a methanotrophic bacterial strain capable of growth on a C1 carbon substrate. The instant bacterial strain may be further characterized by the ability to grow rapidly and efficiently on either methanol or methane as a sole carbon source. This efficiency is due to the presence of a pyrophosphate linked phosphofructokinase enzyme within an operative Embden-Meyerhof pathway. This is a novel observation for methanotrophic bacteria. Functionally, the utilization of the Embden-Meyerhof pathway and pyrophosphate, instead of the Entner-Doudoroff pathway reaction results in highly favorable cellular energetics which is manifested in higher yields, carbon conversion efficiency and growth rate.

The present strain also contains an enzyme system capable of reducing nitrate or nitrite with formation of gaseous nitrogen oxides. This capability is useful for reducing oxygen demand as well as for removing nitrates and nitrites in methane-containing environments such as landfills, wastewater treatment systems or anywhere that methane, oxygen and nitrates are present.

The ability to form large amounts of carbohydrates in the form of starch, polyglucose and/or extracellular polysaccharide is also useful for the production of carbohydrate-based products. Additionally *Methylobacter* 16a is only capable of growth on methane or methanol and is incapable of proliferating in the human body and thus is completely harmless and non-pathogenic. These characteristics make the strain ideally useful for the production of a wide range of products including animal feeds comprising variable carbohydrate/protein ratios.

The strain is shown to be capable of genetic exchange and expression of foreign genes. Additionally the present strain may be identified by the characteristic 16sRNA sequence as set forth in SEQ ID NO:81.

Additionally the present invention provides methods for the production of single cell protein, carbohydrates, and carotenoid pigments, or higher value mixtures of protein, pigments and carbohydrates. Additionally the strain may be used as a denitrifying agent for the



conversion of nitrate or nitrite to nitrous oxide with methane or methanol as carbon source.

Accordingly the invention provides a high growth methanotrophic bacterial strain which:

- 5 (a) grows on a C1 carbon substrate selected from the group consisting of methane and methanol; and
- (b) comprises a functional Embden-Meyerhof carbon pathway, said pathway comprising a gene encoding a pyrophosphate dependent phosphofructokinase enzyme, the gene selected from the group consisting of:
  - 10 (a) an isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO:6;
  - (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS;
  - 15 (c) an isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 437 amino acids that has at least 63% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:6; and
  - 20 (d) an isolated nucleic acid molecule that is complementary to (a), (b) or (c).

- 25 (a) Optionally the present strain may comprise at least one gene encoding a fructose biphosphate aldolase enzyme as part of the functional Embden-Meyerhof carbon pathway. Additionally, the present strain may optionally contain a functional Entner-Doudoroff carbon pathway, where the Entner-Doudoroff carbon pathway comprises at least one gene encoding a keto-deoxy phosphogluconate aldolase.

- 30 (b) In one embodiment the present strain may optionally contain other carbon flux genes encoding polypeptides selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18 and 20.

- 35 (c) In another embodiment the present strain may possess a denitrification pathway where the pathway may optionally comprise genes encoding polypeptides having the amino acid sequences selected from the

group consisting of SEQ ID NO:40, 42, 44, 46, 48, 50, 52, 54, 56, 58 and 60.

In another embodiment the present strain may contain a set of exopolysaccharide synthesizing enzymes where the exopolysaccharide synthesizing enzymes may have the amino acid sequences selected from the group consisting of SEQ ID NO:22, 24, 26, 28, 30, 32, 34, 36, and 38.

In a more specific embodiment the present strain may comprise genes encoding isoprenoid synthesizing enzymes where the enzymes are selected from the group consisting of SEQ ID NO:62, 64, 66, 68, 70, 72, 74, 86, and 78.

In a preferred embodiment the invention provides a method for the production of single cell protein comprising:

- a) contacting the present high growth methanotrophic bacterial strain with a C1 carbon substrate, selected from the group consisting of methane and methanol, in a suitable medium for a time sufficient to permit the expression and accumulation of single cell protein; and
- b) optionally recovering the single cell protein.

It is an additional object of the invention to provide a method for the biotransformation of a nitrogen containing compound selected from the group consisting of ammonia, nitrate, nitrite, and dinitrogen comprising, contacting the present high growth methanotrophic bacterial strain with a C1 carbon substrate selected from the group consisting of methane or methanol, in the presence of the nitrogen containing compound, in a suitable medium for a time sufficient to permit the biotransformation of the nitrogen containing compound.

Similarly it is an object of the present invention to provide a method for the production of a feed product comprising protein, carbohydrates and pigment comprising the steps of:

- a) contacting the high growth methanotrophic bacterial strain of the present invention with a C1 carbon substrate in a suitable medium for a time sufficient to permit the expression and accumulation of the feed product; and
- b) optionally recovering the feed product.

Optionally the relative compositions of protein, carbohydrate and pigment are altered through the up-regulation or down-regulation of any one of the genes encoding the proteins selected from the group consisting

of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, and 69.

In a preferred embodiment the invention provides a method of identifying a high growth methanotrophic bacterial strain comprising:

- 5 (a) growing a sample suspected of containing a high growth methanotrophic bacterial strain on a suitable growth medium in the presence of methane as a sole carbon source;
- (b) identifying colonies that grow under the conditions of step (a);
- 10 (c) analyzing the colonies identified in step (b) for the presence of pyrophosphate dependent phosphofructokinase activity.

#### BRIEF DESCRIPTION OF THE DRAWINGS.

#### SEQUENCE DESCRIPTIONS AND BIOLOGICAL DEPOSITS

Figure 1 shows the growth of *Methylomonas* 16a compared to the growth of *Methylococcus capsulatus* under identical growth conditions.

- 15 Figure 2 is a plot of optical density vs. methanol concentration for a culture of *Methylomonas* 16a grown on methanol alone.

- Figure 3 represents a schematic of the Entner-Doudoroff and Embden-Meyerhof pathways in *Methylomonas* 16a showing microarray expression results numerically ranked in order of decreasing expression level.
- 20

Figure 4 shows oxygen uptake by a cell suspension of *Methylomonas* 16a, in arbitrary units to detect oxygen consumption.

- Figure 5 shows oxygen uptake by a cell suspension of *Methylomonas* 16a, in arbitrary units to detect oxygen consumption before and after sodium nitrite was injected into the incubation.
- 25

Figure 6 is a plot of the concentration of O<sub>2</sub> and N<sub>2</sub>O evolved per hour vs. the concentration of O<sub>2</sub> in the medium of a cell suspension of *Methylomonas* 16a under aerobic conditions.

- The invention can be more fully understood from the following detailed description and the accompanying sequence descriptions which form a part of this application.
- 30

- The following sequence descriptions and sequences listings attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825. The Sequence Descriptions contain the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IYUB standards
- 35

described in *Nucleic Acids Research* 13:3021-3030 (1985) and in the *Biochemical Journal* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in

5 37 C.F.R. §1.822.

Description	SEQ ID Nucleic acid	SEQ ID Peptide
Phosphoglucumutase: carbon Flux	1	2
Glucose 6 phosphate isomerase:Carbon flux	3	4
Phosphofructokinase pyrophosphate dependent: Carbon Flux	5	6
6-Phosphogluconate dehydratase:Carbon flux	7	8
Glucose 6 phosphate 1 dehydrogenase:Carbon Flux	9	10
Transaldolase: Carbon Flux	11	12
Transaldolase: Carbon Flux	13	14
Fructose bisphosphate aldolase:Carbon Flux	15	16
Fructose bisphosphate aldolase:Carbon Flux	17	18
KHG/KDPG Aldolase :Carbon Flux	19	20
<i>ugp</i> : Exopolysaccharaide	21	22
<i>gumD</i> :Exopolysaccharaide	23	24
<i>wza</i> :Exopolysaccharaide	25	26
<i>epsB</i> :Exopolysaccharaide	27	28
<i>epsM</i> :Exopolysaccharaide	30	20
<i>waaE</i> :Exopolysaccharaide	31	32
<i>epsV</i> :Exopolysaccharaide	33	34
<i>gumH</i> :Exopolysaccharaide	35	36
glycosyl transferase:Exopolysaccharaide	37	38
<i>nirF</i> : Denitrification	39	40
<i>nirD</i> : Denitrification	41	42
<i>nirL</i> :Denitrification	43	44
<i>nirG</i> :Denitrification	45	46
<i>nirH</i> :Denitrification	47	48
<i>nirJ</i> :Denitrification	49	50
<i>nasA</i> :Denitrification	51	52
<i>norC</i> :Denitrification	53	54
<i>norB</i> :Denitrification	55	56
<i>norZ</i> :Denitrification	57	58
<i>norS</i> :Denitrification	59	60
<i>dxs</i> :Terpenoid synthesis	61	62

<i>dxr</i> : Terpenoid synthesis	63	64
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Description	SEQ ID Nucleic acid	SEQ ID Peptide
<i>ispF</i> : Terpenoid synthesis	65	66
<i>ispD</i> : Terpenoid synthesis	67	68
<i>pyrG</i> : Terpenoid synthesis	69	70
<i>ispA</i> : Terpenoid synthesis	71	72
<i>ispE</i> : Terpenoid synthesis	73	74
<i>crtN</i> : Terpenoid synthesis	75	76
<i>crtN1</i> : Terpenoid synthesis	77	78
Particulate monooxygenase	79	80
16sRNA for <i>Methylobacter</i> 16a	81	-

Applicants made the following biological deposits under the terms of the Budapest Treaty on the International Recognition of the Deposit of  
5 Micro-organisms for the Purposes of Patent Procedure:

Depositor Identification Reference	International Depository Designation	Date of Deposit
<i>Methylobacter</i> 16a	ATCC PTA 2402	August 21 2000

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention describes the isolation and characterization of  
10 a high growth methanotrophic bacterial strain useful for the production of biomass including proteins, carbohydrates and pigments. The present strain is typed by 16sRNA as a *Methylobacter* sp. and is referred to herein as *Methylobacter* 16a. In addition, the strain may be useful for the production of mixtures of proteins, carbohydrates and pigments for the  
15 purpose of generating animal feeds. The strain possesses the advantage of an active Embden-Meyerhof carbon flux pathway having a pyrophosphate dependent phosphofructokinase gene, which conveys certain energetic advantages to the strain as a production platform for various materials and biomass. Additionally the strain naturally possesses  
20 an active isoprenoid pathway for the generation of pigments indigenous to the strain. In this disclosure, a number of terms and abbreviations are used. The following definitions are provided.

The term "Embden-Meyerhof pathway" refers to the series of biochemical reactions for conversion of hexoses such as glucose and fructose to important cellular 3 carbon intermediates such as glyceraldehyde-3-phosphate, dihydroxyacetone phosphate, phosphophenol pyruvate and pyruvate. These reactions typically proceed with net yield of biochemically useful energy in the form of ATP. The key enzymes unique to the Embden-Meyerhof pathway are phosphofructokinase and fructose-1,6 bisphosphate aldolase.

The term "Entner-Doudoroff pathway" refers to a series of biochemical reactions for conversion of hexoses such as glucose or fructose to important 3 carbon cellular intermediates such as pyruvate and glyceraldehyde-3-phosphate without any net production of biochemically useful energy. The key enzymes unique to the Entner-Doudoroff pathway are the 6-phosphogluconate dehydratase and the ketodeoxyphosphogluconate aldolase.

The term "diagnostic" as it relates to the presence of a gene in a pathway means where a gene having that activity is identified, it is evidence of the presence of that pathway. Within the context of the present invention the presence of a gene encoding a pyrophosphate dependant phosphofructokinase is "diagnostic" for the presence of the Embden-Meyerhof carbon pathway and the presence of gene encoding a ketodeoxyphosphogluconate aldolase is "diagnostic" for the presence of the Entner-Doudoroff carbon pathway.

The term "Yield" is defined herein as the amount of cell mass produced per gram of carbon substrate metabolized.

The term "carbon conversion efficiency" is a measure of how much carbon is assimilated into cell mass and is calculated assuming a biomass composition of  $\text{CH}_2 \text{O}_{0.5} \text{N}_{0.25}$ .

The term "high growth methanotrophic bacterial strain" refers to a bacterium capable of growth with methane or methanol as a sole carbon and energy source and which possesses a functional Embden-Meyerhof carbon flux pathway resulting in a yield of cell mass per gram of C1 substrate metabolized. The specific "high growth methanotrophic bacterial strain" described herein is referred to as "*Methylobacter* 16a" or "16a", which terms are used interchangeably.

The term "a C1 carbon substrate" refers to any carbon-containing molecule that lacks a carbon-carbon bond. Examples are methane,

methanol, formaldehyde, formic acid, methylated amines, and methylated thiols.

The term "functional denitrifying enzymatic pathway" refers to a series of enzymes which sequentially reduce nitrate or nitrite to more reduced products such as nitric oxide, nitrous oxide or ultimately dinitrogen. This process may or may not be energy yielding.

The term "denitrification" refers to the process of converting nitrates or nitrites to gaseous dinitrogen or other gaseous nitrogen oxides. To facilitate denitrification the present strain comprises genes encoding a number of enzymes in the denitrification pathway including: the *nir* genes (*nirD*, *nirF*, *nirG*, *nirH*, *nirJ*, *nirL* and *nirS*) encoding the nitrite reductase which catalyzes the reduction of nitrite ( $\text{NO}_2$ ) to nitric oxide, the *nasA* gene, encoding nitrate reductase which catalyzes the reduction of nitrate ( $\text{NO}_3$ ) to nitrite ( $\text{NO}_2$ ); and the *nor* genes (*norB*, *norC* or *norZ*) encoding a nitric oxide reductase which catalyzes the reduction of nitric oxide ( $\text{NO}$ ) to nitrous oxide ( $\text{N}_2\text{O}$ ).

The term "isoprenoid compound" refers to any compound which is derived via the pathway beginning with isopentenyl pyrophosphate and formed by the head to tail condensation of isoprene units which may be of 5, 10, 15, 20, 30 or 40 carbons in length. The term "isoprenoid pigment" refers to a class of compounds which typically have strong light absorbing properties and are derived from the head to tail condensation of 5, 10, 15, 20, 25, 30 or 40 carbon isoprene chains. These isoprene chains are ultimately derived from isopentenyl pyrophosphate. A number of genes and gene products are associated with the present strain encoding the isoprenoid biosynthetic pathway including the *dxs* gene, encoding 1-deoxyxylulose-5-phosphate synthase, the *dxr* gene, encoding 1-deoxyxylulose-5-phosphate reductoisomerase, the "*ispD*," gene encoding the 2C-methyl-D-erythritol cytidyltransferase enzyme, the "*ispE*" gene encoding the 4-diphosphocytidyl-2-C-methylethritol kinase, the "*ispF*" gene encoding a 2C-methyl-d-erythritol 2,4-cyclodiphosphate synthase, the "*pyrG*" gene, encoding a CTP synthase, the "*ispA*" gene, encoding geranyltransferase or farnesyl diphosphate synthase and the "*ctrN*" and "*ctrN 1*" genes, encoding diapophytoene dehydrogenase.

The term "single cell protein" will be abbreviated "SCP" and refers to a protein derived from organisms that exist in the unicellular, or single cell,



state. This includes unicellular bacteria, yeasts, fungi or eukaryotic single cell organisms such as algae.

The term "extracellular polysaccharide" or "exocellular polysaccharide" will be abbreviated "ESP" and refers to a polysaccharide produced by methanotrophic bacteria typically comprising a carbohydrate "backbone" polymer as cross-linking carbohydrate polymers. These polymers are excreted on the outside of the microbial cell and may function in adhesion to surfaces or as a response to environmental stress. The present strain comprises a number of genes encoding various steps in the synthesis of extracellular polysaccharide including the "*ugp*" gene encoding UDP-glucose pyrophosphorylase, the "*gumD*" and "*waaE*" genes encoding glycosyltransferases, the "*wza*" and "*epsB*" genes, encoding polysaccharide export proteins, the "*epsM*" gene, encoding a polysaccharide biosynthesis related protein, and the "*epsV*" gene, encoding a sugar transferase.

The term "carbohydrate" refers to any sugar containing constituent, particularly storage forms, such as glycogen or starch and extracellular polysaccharides.

The term "fermentation product" refers to products derived from the fermentation of any carbohydrate formed by the methanotrophic bacterium from methane or methanol.

The term "particulate methane monooxygenase" will be abbreviated as "pMMO" and will refer to a membrane-associated methane monooxygenase which inserts oxygen in to the enzyme substrate.

The terms "soluble methane monooxygenase" will be abbreviated as "sMMO" and will refer to a soluble or cytoplasmic methane monooxygenase - localized in the cytoplasm.

As used herein, an "isolated nucleic acid fragment" is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. An isolated nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers to any gene that is not a

native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure.

The term "percent identity", as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in: Computational Molecular Biology (Lesk, A. M., ed.) Oxford University Press, NY (1988); Biocomputing: Informatics and Genome Projects (Smith, D. W., ed.) Academic Press, NY (1993); Computer Analysis of Sequence Data, Part I (Griffin, A. M., and Griffin, H. G., eds.) Humana Press, NJ (1994); Sequence Analysis in Molecular Biology (von Heinje, G., ed.) Academic Press (1987); and Sequence Analysis Primer (Gribskov, M. and Devereux, J., eds.) Stockton Press, NY (1991). Preferred methods to determine identity are designed to give the best match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Sequence alignments and percent identity calculations may be performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

Suitable nucleic acid fragments (isolated polynucleotides of the present invention) encode polypeptides that are at least about 70% identical, preferably at least about 80% identical to the amino acid sequences reported herein. Preferred nucleic acid fragments encode amino acid sequences that are about 85% identical to the amino acid sequences reported herein. More preferred nucleic acid fragments encode amino acid sequences that are at least about 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are at least about 95% identical to the amino acid sequences reported herein. Suitable nucleic acid fragments not only have the above homologies but typically encode a polypeptide having at least 50 amino acids, preferably at least 100 amino acids, more preferably at least 150 amino acids, still more preferably at least 200 amino acids, and most preferably at least 250 amino acids.

A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength. Hybridization and washing conditions are well known and exemplified in Sambrook, J., Fritsch, E. F. and Maniatis, T. Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989), particularly Chapter 11 and Table 11.1 therein (entirely incorporated herein by reference). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. Stringency conditions can be adjusted to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions uses two final washes in 0.1X SSC, 0.1% SDS

at 65°C. An additional preferred set of stringent conditions include 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS).

Hybridization requires that the two nucleic acids contain  
5 complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two  
10 nucleotide sequences, the greater the value of  $T_m$  for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher  $T_m$ ) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating  $T_m$  have been derived  
15 (see Sambrook et al., *supra*, 9.50-9.51). For hybridizations with shorter nucleic acids, i.e., oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., *supra*, 11.7-11.8). In one embodiment the length for a hybridizable nucleic acid is at least about 10 nucleotides.  
20 Preferable a minimum length for a hybridizable nucleic acid is at least about 15 nucleotides; more preferably at least about 20 nucleotides; and most preferably the length is at least 30 nucleotides. Furthermore, the skilled artisan will recognize that the temperature and wash solution salt concentration may be adjusted as necessary according to factors such as  
25 length of the probe.

The term "sequence analysis software" refers to any computer algorithm or software program that is useful for the analysis of nucleotide or amino acid sequences. "Sequence analysis software" may be commercially available or independently developed. Typical sequence  
30 analysis software will include but is not limited to the GCG suite of programs (Wisconsin Package Version 9.0, Genetics Computer Group (GCG), Madison, WI), BLASTP, BLASTN, BLASTX (Altschul et al., *J. Mol. Biol.* 215:403-410 (1990), and DNASTAR (DNASTAR, Inc. 1228 S. Park St. Madison, WI 53715 USA), and the FASTA program incorporating the  
35 Smith-Waterman algorithm (W. R. Pearson, *Comput. Methods Genome Res.*, [Proc. Int. Symp.] (1994), Meeting Date 1992, 111-20. Editor(s): Suhai, Sandor. Publisher: Plenum, New York, NY). Within the context of

this application it will be understood that where sequence analysis software is used for analysis, that the results of the analysis will be based on the "default values" of the program referenced, unless otherwise specified. As used herein "default values" will mean any set of values or parameters which originally load with the software when first initialized.

Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by Sambrook, J., Fritsch, E. F. and Maniatis, T., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989) (hereinafter "Maniatis"); and by Silhavy, T. J., Bennis, M. L. and Enquist, L. W., Experiments with Gene Fusions, Cold Spring Harbor Laboratory Cold Press Spring Harbor, NY (1984); and by Ausubel, F. M. et al., Current Protocols in Molecular Biology, published by Greene Publishing Assoc. and Wiley-Interscience (1987).

The present invention provides a unique methanotrophic bacterial strain, useful for the production of a variety materials from C1 carbon sources such as methane and methanol. The strain is referred to herein as *Methylomonas* 16a, and is characterized by rapid doubling time, high yield and the presence of genes encoding both the Entner-Doudoroff carbon pathway as well as the Embden-Meyerhof pathway, allowing for versatility in carbon flux management and higher efficiency of carbon incorporation. The strain has been shown to produce a variety of food and feed products such as single cell protein, exopolysaccharide and starch. The strain has particularly high value in the production of food and feed materials as it is possible to manipulate the various concentrations of protein, carbohydrate and starch all within the same organism. This capability will permit strains to be uniquely tailored for individual specific food and feed applications. Additionally the strain has demonstrated utility in the production of terpenoid and carotenoid compounds, useful as pigments and as monomers in polymeric materials.

#### Isolation of *Methylomonas* 16a

The original environmental sample containing *Methylomonas* 16a was obtained from pond sediment. The pond sediment was inoculated directly into a defined mineral medium under 25% methane in air. Methane was used as the sole source of carbon and energy. Growth was followed until the optical density at 660 nm was stable, whereupon the culture was transferred to fresh medium such that a 1:100 dilution was achieved. After

3 successive transfers with methane as the sole carbon and energy source the culture was plated onto defined minimal medium agar and incubated under 25% methane in air. Many methanotrophic bacterial species were isolated in this manner. However, *Methylomonas* 16a was selected as the organism to study due to the rapid growth of colonies, large colony size, its ability to grow on minimal media, and pink pigmentation indicative of an active biosynthetic pathway for carotenoids.

Methanotrophs are classified into three metabolic groups ("Type I", "Type X" or "Type II") based on the mode of carbon incorporation, morphology, %GC content and the presence or absence of key specific enzymes. Example 4, Table 2 shows key traits determined for *Methylomonas* 16a in relation to the three major groupings of methanotrophs. The strain clearly falls into the Type I grouping based on every trait, with the exception of nitrogen fixation. It is generally well accepted that these organisms do not fix nitrogen. Therefore, *Methylomonas* 16a appears unique in this aspect of nitrogen metabolism.

16SrRNA extracted from the strain was sequenced and compared to known 16SrRNAs from other microorganisms. The data showed 96% identity to sequences from *Methylomonas* sp. KSP III and *Methylomonas* sp. strain LW13. Based on this evidence, as well as the other physiological traits described in Table 2 (Example 4), it was concluded that the strain was a member of the genus *Methylomonas*.

#### Metabolic and Physiological Characterization of *Methylomonas* 16a

Carbon Metabolism: The present methanotrophic bacterial strain, *Methylomonas* 16a, converts methane to methanol via a methane monooxygenase as the first step in carbon utilization. The methane monooxygenase present in the strain is a particulate, as opposed to a soluble, monooxygenase. Particulate methane monooxygenases (pMMO) are well known in the art (Murrell et al., *Arch. Microbiol.* (2000), 173(5-6), 325-332) and many have been isolated and sequenced. pMMO's are characterized by their narrow substrate specificity as opposed to sMMO's which are less discriminating. For this reason the pMMO enzyme is favored for the production of bulk chemicals since the sMMO is likely to modify many of the chemical intermediates needed for the efficient production of a specific product.

The gene and gene product corresponding to the pMMO isolated from the present strain have been sequenced and functionally identified on

the basis of homology comparisons to sequences in publicly available databases. The instant sequence is highly homologous to that isolated from *Methylococcus capsulatus* (GenBank B57266).

The present strain contains several anomalies in the carbon utilization pathway. For example, based on genome sequence data, the strain is shown to contain genes for two pathways of hexose metabolism. The Entner-Doudoroff Pathway utilizing the keto-deoxy phosphogluconate aldolase enzyme is present in the strain. Is generally well accepted that this is the operative pathway in obligate methanotrophs. Also present, however, is the Embden-Meyerhof pathway which utilizes the fructose bisphosphate aldolase enzyme. It is well known that this pathway is either not present or not operative in obligate methanotrophs. Energetically, the latter pathway is most favorable and allows greater yield of biologically useful energy, ultimately resulting in greater yield production of cell mass and other cell mass-dependent products in *Methylomonas* 16a. The activity of this pathway in the present 16a strain has been confirmed through microarray data and biochemical evidence measuring the reduction of ATP. Although the 16a strain has been shown to possess both the Embden-Meyerhof and the Entner-Doudoroff pathway enzymes the data suggests that the Embden-Meyerhof pathway enzymes are more strongly expressed than the Entner-Doudoroff pathway enzymes. This result is surprising and counter to existing beliefs concerning the glycolytic metabolism of methanotrophic bacteria. Applicants have discovered other methanotrophic bacteria having this characteristic, including for example, *Methylomonas clara* and *Methylosinus sporium*. It is likely that this activity has remained undiscovered in methanotrophs due to the lack of activity of the enzyme with ATP, the typical phosphoryl donor for the enzyme in most bacterial systems.

A particularly novel and useful feature of the Embden-Meyerhof pathway in strain 16a is that the key phosphofructokinase step is pyrophosphate dependent instead of ATP dependent. This feature adds to the energy yield of the pathway by using pyrophosphate instead of ATP (Example 6). Because of its significance in providing an energetic advantage to the strain, this gene in the carbon flux pathway is considered diagnostic for the present strain.

Comparison of the pyrophosphate dependent phosphofructokinase gene sequence (SEQ ID NO:5) and deduced amino acid sequence (SEQ

ID NO:6) to public databases reveals that the most similar known sequences is about 63% identical to the amino acid sequence reported herein over a length of 437 amino acid using a Smith-Waterman alignment algorithm (W. R. Pearson, *Comput. Methods Genome Res.*, [Proc. Int. Symp.] (1994), Meeting Date 1992, 111-20. Editor(s): Suhai, Sandor. Publisher: Plenum, New York, NY). More preferred amino acid fragments are at least about 80%-90% identical to the sequences herein. Most preferred are nucleic acid fragments that are at least 95% identical to the amino acid fragments reported herein. Similarly, preferred pyrophosphate dependent phosphofructokinase encoding nucleic acid sequences corresponding to the instant gene are those encoding active proteins and which are at least 80% identical to the nucleic acid sequences of reported herein. More preferred pyrophosphate dependent phosphofructokinase nucleic acid fragments are at least 90% identical to the sequences herein. Most preferred are pyrophosphate dependent phosphofructokinase nucleic acid fragments that are at least 95% identical to the nucleic acid fragments reported herein.

Accordingly the invention provides a high growth methanotrophic bacterial strain which:

- (a) grows on a C1 carbon substrate selected from the group consisting of methane and methanol; and
- (b) comprises a functional Embden-Meyerhof carbon pathway, said pathway comprising a gene encoding a pyrophosphate dependent phosphofructokinase enzyme, the gene selected from the group consisting of:
  - (a) an isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO:6;
  - (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS;
  - (c) an isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 437 amino acids that has at least 63% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:6; and



(d) an isolated nucleic acid molecule that is complementary to (a), (b) or (c).

Methane and methanol are the only substrates shown to support growth of *Methylomonas* 16a. The strain is grown on defined medium without the addition of complex growth factors. Methanol utilization is reported to typically require "adaptation" and growth on methanol concentration ranging from 0.1% to 3% is also reported as "variable". *Methylomonas* 16a was shown to grow on methanol concentrations as high as 600 mM (2.4%) without adaptation and with good yield. (Figure 2).

In methanotrophic bacteria methane is converted to biomolecules via a cyclic set of reactions known as the ribulose monophosphate pathway or RuMP cycle. This pathway is comprised of three phases, each phase being a series of enzymatic steps. The first step is "fixation" or incorporation of C-1 (formaldehyde) into a pentose to form a hexose or six carbon sugar. This occurs via a condensation reaction between a 5 carbon sugar (pentose) and formaldehyde and is catalyzed by hexulose monophosphate synthase. The second phase is termed "cleavage" and results in splitting of that hexose into two 3 carbon molecules. One of those three carbon molecules is recycled back through the RuMP pathway and the other 3 carbon fragment is utilized for cell growth. In methanotrophs and methylotrophs the RuMP pathway may occur as one of three variants. However, only two of these variants are commonly found: the FBP/TA (fructose biphosphotase/Transaldolase) or the KDPG/TA (keto deoxy phosphogluconate/transaldolase) pathway. (Dijkhuizen L., G.E. Devries. The physiology and biochemistry of aerobic methanol-utilizing gram negative and gram positive bacteria. In: Methane and Methanol Utilizers 1992, ed. Colin Murrell and Howard Dalton. Plenum Press, NY).

The present strain is unique in the way it handles the "cleavage" steps as genes were found that carry out this conversion via fructose biphosphate as a key intermediate. The genes for fructose biphosphate aldolase and transaldolase were found clustered together on one piece of DNA. Secondly the genes for the other variant involving the keto deoxy phosphogluconate intermediate were also found clustered together. Available literature teaches that these organisms (methylotrophs and methanotrophs) rely solely on the KDPG pathway and that the FBP-dependent fixation pathway is utilized by facultative methylotrophs

(Dijkhuizen et al., *supra*). Therefore the latter observation is expected, whereas the former is not. The finding of the FBP genes in an obligate methane utilizing bacterium is both surprising and suggestive of utility. The FBP pathway is energetically favorable to the host microorganism due to the fact that less energy (ATP) is utilized than is utilized in the KDPG pathway. Thus organisms that utilize the FBP pathway may have an energetic advantage and growth advantage over those that utilize the KDPG pathway. This advantage may also be useful for energy-requiring production pathways in the strain. By using this pathway, a methane-utilizing bacterium may have an advantage over other methane utilizing organisms as production platforms for either single cell protein or for any other product derived from the flow of carbon through the RuMP pathway.

Accordingly the present invention provides a *Methylobacter* having two distinct carbon flux pathways, comprising genes and gene products as set forth in SEQ ID NO:1-20, and encoding both a pyrophosphate dependent phosphofructokinase pyrophosphate and a keto-deoxy phosphogluconate (KDPG) aldolase. Comparison of the KDPG aldolase gene sequence (SEQ ID NO:19) and deduced amino acid sequence (SEQ ID NO:20) to public databases reveals that the most similar known sequences is about 59% identical to the amino acid sequence of reported herein over a length of 212 amino acid using a Smith-Waterman alignment algorithm (W. R. Pearson, *supra*). More preferred amino acid fragments are at least about 80%-90% identical to the sequences herein. Most preferred are nucleic acid fragments that are at least 95% identical to the amino acid fragments reported herein. Similarly, preferred KDPG aldolase encoding nucleic acid sequences corresponding to the instant gene are those encoding active proteins and which are at least 80% identical to the nucleic acid sequences of reported herein. More preferred KDPG aldolase nucleic acid fragments are at least 90% identical to the sequences herein. Most preferred are KDPG aldolase nucleic acid fragments that are at least 95% identical to the nucleic acid fragments reported herein.

It is thus an object of the invention to provide a high growth methanotrophic bacterial strain having the ability to grow exclusively on either methane or methanol, comprising a functional Embden-Meyerhof carbon pathway, said pathway comprising a gene encoding a pyrophosphate dependent phosphofructokinase enzyme and at least one

gene encoding a keto-deoxy phosphogluconate aldolase enzyme, selected from the group consisting of:

- (a) an isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO:20;
- 5 (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS;
- 10 (c) an isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 212 amino acids that has at least 59% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:20; and
- 15 (d) an isolated nucleic acid molecule that is complementary to (a), (b) or (c).

In addition to the pyrophosphate dependent phosphofructokinase enzyme and keto-deoxy phosphogluconate aldolase enzyme, the strain comprises other carbon flux genes including an FBP aldolase, phosphoglucomutase, pyrophosphate dependent phosphofructokinase pyrophosphate, 6-Phosphogluconate dehydratase, and a glucose-6 phosphate-1 dehydrogenase. The phosphoglucomutase is responsible for the interconversion of glucose-6-phosphate to glucose-1-phosphate, which feeds into either the Entner-Doudoroff or Embden-Meyerhof carbon flux pathways. As shown in Figure 3, fructose-6-phosphate may be convert to either glucose-6-phosphate by glucose phosphate isomerase (Entner-Doudoroff) or to fructose-1,6-bisphosphate (FBP) by a phosphofructokinase (Embden-Meyerhof). Following the Embden-Meyerhof pathway, FBP is then taken to two three-carbon moieties (dihydroxyacetone and 3-phosphoglyceraldehyde) by the FBP aldolase. Returning to the Entner-Doudoroff system, glucose-6-phosphate is taken to 6-phosphogluconate by a glucose-6-phosphate dehydrogenase which is subsequently taken to 2-keto-3-deoxy-6-phosphogluconate (KDPG) by a 6 phosphogluconate dehydratase. The KDPG is then converted to two three-carbon moieties (pyruvate and 3-phosphoglyceraldehyde) by a KDPG aldolase. Thus the Embden-Meyerhof and Entner-Doudoroff pathways are rejoined at the level of 3-phosphoglyceraldehyde.

### Identification of High Growth Methanotrophic Bacteria

Although the present 16a strain has been isolated fortuitously, it is contemplated that the present teaching will enable the general identification and isolation of similar strains. For example, the key characteristics of the present high growth strain are that it is an obligate methanotroph, using only either methane or methanol as a sole carbon source; and it possesses a functional Embden-Meyerhof pathway, and particularly a gene encoding a pyrophosphate dependent phosphofructokinase. Methods for the isolation of methanotrophs are common and well known in the art (See for example Thomas D. Brock in Biotechnology: A Textbook of Industrial Microbiology, Second Edition (1989) Sinauer Associates, Inc., Sunderland, MA., or Deshpande, Mukund V., *Appl. Biochem. Biotechnol.*, 36, 227, (1992)). Similarly pyrophosphate dependent phosphofructokinase has been well characterized in mammalian systems and assay methods have been well developed (see for example Schliselfeld et al. *Clin. Biochem.* (1996), 29(1), 79-83; Clark et al., *J. Mol. Cell. Cardiol.* (1980), 12(10), 1053-64). The contemporary microbiologist will be able to use these techniques to identify the present high growth strain.

The specific strain of the present invention possesses a specific pyrophosphate dependent phosphofructokinase having the amino acid sequence as set forth in SEQ ID NO:6. The present strain may be further characterized by analyzing a methanotrophic bacterial strain for the presence of the gene encoding this enzyme.

It is therefore an object of the invention to provide a method of identifying a high growth methanotrophic bacterial strain comprising:

- (a) growing a sample suspected of containing a high growth methanotrophic bacterial strain on a suitable growth medium in the presence of methane as a sole carbon source;
- (b) identifying colonies that grow on the conditions of step (a);
- (c) analyzing the colonies identified in step (b) for the presence of pyrophosphate dependent phosphofructokinase activity.

Growth Characteristics: The presence of the above mentioned carbon flux characteristics was previously unknown in methanotrophic bacteria and may explain the rapid growth rate and the increased carbon conversion efficiency of this strains and other strains possessing this pathway, relative to strain that do not have this pathway. The present *Methylomonas* 16a has been shown to grow on methane with a doubling time of only 2.5 h. This is a very high growth rate

and is an obvious advantage for commercial use as well as for the genetic manipulations performed in development of the strain. Additionally, *Methylomonas* has no requirement for organic growth factors such as yeast extract or other costly fermentation additives. The strain requires only methane or methanol, inorganic minerals, oxygen and water for optimum growth, giving the present strain an advantage for large scale growth at low cost.

Particularly noteworthy is the high yield of the present strain. Yield is defined herein as the amount of cell mass produced per gram of carbon substrate metabolized. The present strain has shown the ability to produce greater than 0.8 and preferably greater than 1.0 grams of cell mass per gram of methane metabolized. Similarly the present strain has shown the ability to produce greater than 0.30 and preferably greater than 0.45, more preferably greater than 0.5 cell mass per gram of methanol metabolized.

Carbon conversion efficiency is another measure of how much carbon is assimilated into cell mass. Carbon conversion efficiency is expressed in units of g/mol methane ( 1 g dry wt/g methane) / g/ mol biomass. Carbon conversion efficiency is calculated assuming a biomass composition of  $\text{CH}_2\text{O}_{0.5}\text{N}_{0.25}$ . The present strain will have a particularly high carbon conversion efficiency where an efficiency of greater than 40 is common, an efficiency of greater than 50 is preferred, a conversion of greater than 65 is highly preferred and an efficient of greater than 70 is most preferred.

Methanol Utilization: *Methylomonas* 16a is shown to grow at methanol concentrations as high as 600 mM . Typically methanol can be toxic at these concentrations to some methanotrophic bacteria. *Methylomonas* 16a can tolerate up to about 2.4% methanol which is at the upper end of the known spectrum of methanol tolerance for methanotrophic bacteria (Green, Peter, Taxonomy of Methylophilic Bacteria. In: Methane and Methanol Utilizers (Biotechnology Handbooks 5) J. Colin Murrell and Howard Dalton eds., 1992 Plenum Press NY, pp 23-84). This feature again allows for much lower capital costs in reactor design since tolerance for methanol is higher necessitating reactors with fewer mixing ports (i.e. lower construction costs). This issue (high reactor costs due to mixing requirements to overcome methanol toxicity) is a major drawback to growth of methanotrophic bacteria on methanol.

Glycogen Production: *Methylomonas* 16a has been shown to produce in excess of 50% of its weight as glycogen during active growth on methanol and significant amounts of glycogen during active (non-stress associated) growth on methane. This aspect is useful for the production of mixtures of protein and

carbohydrate to serve a wider array of animal feed nutritional needs as compared to other obligate methanotrophs producing only protein as the sole product. Alternatively, this trait enables *Methylobacter* 16a to serve as a host strain for the production of glycogen from methane or methanol. Furthermore, internal  
5 hexose metabolism is clearly occurring in *Methylobacter* 16a. Thus the organism can serve as host for the production of chemical products typically considered to be only produced by carbohydrate metabolism. Accordingly the invention provides a *Methylobacter* strain having the ability to produce in excess of 50% of its weight of glycogen when grown on methanol, where about 20% to  
10 about 40% is typical.

Pigment and Terpenoid Production: The present *Methylobacter* strain is useful for the production of a variety of pigments and particularly the isoprenoid pigments. This class of pigments are known to have strong light absorbing properties and are derived from the head to tail  
15 condensation of 5, 10, 15, 20, 25, 30 or 40 carbon isoprene chains. One specific pigment identified in the present strain is a C-30 carotenoid. The content of this pigment is very high in the cell and is indicative of naturally high carbon flow through the isoprenoid pathway. This aspect provides the basis for viewing the isoprenoid pathway as a "backbone production  
20 pathway" for isoprenoid-derived products. It is contemplated for example that high value carotenoids such as astaxanthin,  $\beta$ -carotene, canthaxanthin, and lutein may be produced by the instant organism.

Additionally the present strain is expected to have the ability to produce various isoprenoid compounds. Isoprenoids are an extremely  
25 large and diverse group of natural products that have a common biosynthetic origin based on a single metabolic precursor known as isopentenyl diphosphate (IPP). The group of natural products known as isoprenoids includes all substances that are derived biosynthetically from the 5-carbon compound isopentenyl diphosphate. Isoprenoid compounds  
30 are also referred to as "terpenes" or "terpenoids", which is the term used in the designation of the various classes of these examples (Spurgeon and Porter, Biosynthesis of Isoprenoid Compounds, pp 3-46, A Wiley-Interscience Publication (1981)). Isoprenoids are ubiquitous compounds found in all living organisms. Some of the well-known examples of  
35 isoprenoids are steroids (triterpenes), carotenoids (tetraterpenes), and squalene, just to name a few.

The biosynthesis of such compounds typically involve the enzyme isopentenyl pyrophosphate and are formed by the head to tail condensation of isoprene units which may be of 5, 10, 15, 20, 30 or 40 carbons in length.

5 It is contemplated that other, related, small cyclic molecules such as limonene, menthol and geraniol may be produced in the present strain via the introduction of the appropriate plant-derived terpene synthases. Thus the isoprenoid pathway may be viewed as a platform pathway for production of complex cyclic and unsaturated molecules from methane or  
10 methanol. This capability is unique to biology, purely chemical processes cannot convert C-1 compounds to cyclic molecules with any degree of specificity.

Many steps in isoprenoid pathways are known. For example, the initial steps of the alternate pathway involve the condensation of 3-carbon  
15 molecules (pyruvate and C1 aldehyde group, D-glyceraldehyde 3-Phosphate), to yield a 5-carbon compound (D-1-deoxyxylulose-5-phosphate). Lois et al. has reported a gene, *dxs*, that encodes D-1-deoxyxylulose-5-phosphate synthase (DXS) that catalyzes the synthesis of D-1-deoxyxylulose-5-phosphate in *E. coli* (*Proc. Natl. Acad. Sci. USA* 95:  
20 2105-2110 (1998)).

Next, the intramolecular rearrangement of D-1-deoxyxylulose-5-phosphate occurs by an unspecified reduction process for the formation of 2-C-methyl-D-erythritol-4-phosphate. One of the enzymes involved in the reduction process is D-1-deoxyxylulose-5-phosphate reductoisomerase  
25 (DXR). Takahashi et al. reported the *dxr* gene product catalyzes the formation of 2-C-methyl-D-erythritol-4-phosphate in the alternate pathway in *E. coli* (*Proc. Natl. Acad. Sci. USA* 95: 9879-9884 (1998)).

Steps converting 2-C-methyl-D-erythritol-4-phosphate to isopentenyl monophosphate are not well characterized although some  
30 steps are known. 2-C-methyl-D-erythritol-4-phosphate is converted into 4-diphosphocytidyl-2C-methyl-D-erythritol in a cytosine triphosphate (CTP) dependent reaction by the enzyme encoded by non-annotated gene *ygbP*, encoding a 2C-methyl-d-erythritol cytidyltransferase. Rondich et al. reported a YgbP protein in *E. coli* that catalyzes the reaction mentioned  
35 above (*Proc. Natl. Acad. Sci. USA* 96:11758-11763 (1999)). Recently, *ygbP* gene was renamed as *ispD* as a part of the *isp* gene cluster. The 2 position hydroxy group of 4-diphosphocytidyl-2C-methyl-D-erythritol can

be phosphorylated in an ATP dependent reaction by a 4-diphosphocytidyl-2-C-methylerythritol kinase encoded by the *ychB* gene. Luttgen et al. has reported a YchB protein in *E. coli* that phosphorylates 4-diphosphocytidyl-2C-methyl-D-erythritol, resulting in 4-diphosphocytidyl-2C-methyl-D-erythritol 2-phosphate (*Proc. Natl. Acad. Sci. USA* 97:1062-1067 (2000)).  
5 Recently, the *ychB* gene was renamed as *ispE* as a part of the *isp* gene cluster.

Herz et al. reported that the *ygbB* gene product (2C-methyl-d-erythritol 2,4-cyclodiphosphate synthase) in *E. coli* converts 4-diphosphocytidyl-2C-methyl-D-erythritol 2-phosphate to 2C-methyl-D-erythritol 2,4-cyclodiphosphate in a CTP dependent reaction. 2C-methyl-D-erythritol 2,4-cyclodiphosphate can be further converted into carotenoids through the carotenoid biosynthesis pathway (*Proc. Natl. Acad. Sci. USA* 97:2486-2490 (2000)). Recently, the *ygbB* gene was renamed as *ispF* as  
15 a part of *isp* gene cluster.

Both reactions catalyzed by the YgbB and YgbP enzymes are carried out in CTP dependent manner. Thus CTP synthase plays an important role in the isoprenoid pathway. PyrG encoded by the *pyrG* gene in *E. coli* was determined to encode CTP synthase (Weng et al., *J. Biol. Chem.*, 261:5568-5574 (1986)).  
20

Following several reactions not yet characterized, isopentenyl monophosphate is formed. Isopentenyl monophosphate is converted to an isopentenyl diphosphate (IPP) by isopentenyl monophosphate kinase enzyme encoded by the *ipk* gene (Lange and Croteau, *Proc. Natl. Acad. Sci. USA* 96:13714-13719 (1999)).  
25

Prenyltransferases constitute a broad group of enzymes catalyzing the consecutive condensation of isopentenyl diphosphate (IPP), resulting in the formation of prenyl diphosphates of various chain lengths. Homologous genes of prenyl transferase have highly conserved regions in their amino acid sequences. Ohto et al. reported three prenyl transferase genes in cyanobacterium *Synechococcus elongatus* (*Plant Mol. Biol.* 40:307-321 (1999)). They are geranylgeranyl (C20) diphosphate synthase, farnesyl (C15) diphosphate synthase (*ispA*), and another prenyltransferase that can catalyze the synthesis of five prenyl  
30 diphosphates of various length.  
35

Further down in the isoprenoid biosynthesis pathway, more genes are involved in the synthesis of specific isoprenoids. As an example, the



*crtN* gene that was found in *Heliobacillus mobilis* (*Proc. Natl. Acad. Sci. USA* 95:14851-14856 (1998)) encodes a diapophytoene dehydrogenase that is a part of the carotenoid biosynthesis pathway.

Although some of the genes involved in isoprenoid pathways are well known, the presence of genes involved in the isoprenoid pathway of *Methylobacillus* *sp.* is rare. It is surprising therefore to find all of the above mentioned genes in the present strain (SEQ ID NO:61-SEQ ID NO:78). Tgus suggests that the present strain will be useful for the production of a variety of terpenoids. Accordingly the invention provides a *Methylobacillus* strain having the genes and gene products as set forth in SEQ ID NO:61-SEQ ID NO:78, encoding a D-1-deoxyxylulose-5-phosphate synthase, a D-1-deoxyxylulose-5-phosphate reductoisomerase, 2C-methyl-d-erythritol 2,4-cyclodiphosphate synthase, a 2C-methyl-d-erythritol cytidyltransferase, a CTP synthase, a Geranyltranstransferase (also farnesyl-diphosphate synthase), a 4-diphosphocytidyl-2-C-methylerythritol kinase, and a diapophytoene dehydrogenase.

Production of Single Cell Protein: The present strain is useful for the production of single cell protein (SCP) which has value in the food and feed industries. Methods for the use of methanotrophs as production platforms for the production of SCP are well known in the art (see for example US 4,795,708; Shojaosadati et al., *Amirkabir* (1996), 8(30), 33-41). The present strain is well suited for this application due to its advantages in carbon flux and reduced oxygen consumption in the presence of a nitrogen source. The strain is well suited for the production of single cell protein under either aerobic or anaerobic conditions.

The present strain compares favorably with other known strains, producing up to about 1.3 g protein/dry weight/ g methane and up to about 0.45 g protein/dry weight/ g methanol.

Production of exopolysaccharides: Polysaccharides are sugar polymers that have been used widely as a thickener in food and non-food industries (Sanford et al. *Pure & Appl. Chem.* 56: 879-892 (1984); Sutherland, *Trends Biotechnol.* 16(1): 41-6 (1998)). They can be found in food products such as salad dressing, jam, frozen food, bakery products, canned food and dry food. Many other applications include suspending agents for pesticides, paints and other coating agents. They can act as flocculent, binders, film-formers, lubricants and friction reducers.

Furthermore, exopolysaccharides are commonly used in the oil field for oil recovery.

Traditionally, industrially useful polysaccharides have been derived from algal and plant sources. Over the past decade polysaccharides  
5 derived from microbes have been found increased usage (Sanford et al. *Pure & Appl. Chem.* 56: 879-892 (1984); Sutherland, *Trends Biotechnol.* 16(1): 41-6 (1998)).

Many other genes involved in exopolysaccharide biosynthesis have been characterized or sequenced from other organisms. The *epsB* gene  
10 encodes the *EpsB* protein that is probably involved in polymerization and/or export of *EPS*, and has been sequenced in *Ralstonia sola* (Huang et al, *Mol. Microbiol.* 16: 977-989 (1995)). The *espM* gene encoding the *EspM* protein has been found in the *esp* gene cluster from *Streptococcus thermophilus* (Stingele et al, *J. Bacteriol.* 178: 1680-1690 (1996)). Another  
15 putative polysaccharide export protein, WZA, is identified in *E. coli*. (Blattner et al., *Science* 277: 1453-1474 (1997)). Finally, the *epsV* gene encodes the *EpsV* protein, a transferase which transfers the sugar to polysaccharide intermediates, and it has also been sequenced in  
20 *Streptococcus thermophilus* (Bourgoin et al., *Plasmid* 40: 44-49 (1998); Bourgoin, F., et al., *Gene* 233:151-161 (1999)).

In spite of the abundance of information regarding genes encoding microbial exopolysaccharides, no genes involved in this pathway have been isolated or characterized from C1 utilizing organisms, such as  
25 *Methylobacter*. As noted above, microbial exopolysaccharides have a variety of uses and it would be an advantage to synthesize this material from an abundant and inexpensive carbon source such as methane.

Surprisingly, the present *Methylobacter* 16a has been shown to produce extrapolsaccharides at high levels. The genes encoding the relevant polysaccharide synthesis pathways have been isolated and  
30 characterized and are described along with their gene products in SEQ ID NO:21-SEQ ID NO:38.

Accordingly, the present invention provides a *Methylobacter* strain having the ability to synthesize exopolysaccharides and having genes encoding the *ugp*, *gumD*, *wza*, *epsB*, *epsM*, *waaE*, *epsV*, *gumH* and  
35 glycosyl transferase proteins associated with microbial polysaccharide biosynthesis.

Denitrification: The presence of denitrification enzymes in obligate methanotrophs is unknown. The present strain contains a pathway comprised of genes and gene products as set forth in SEQ ID NO:39-SEQ ID NO:60. A novel feature of the present *Methylomonas* 16a is the ability to utilize a nitrogen source at low oxygen tensions as an additional "electron sink" for reducing equivalents derived from methane or methanol. Nitrogen sources may include, but are not limited to, nitrite, nitrate, ammonium and dinitrogen. The strain is shown to reduce nitrate or nitrite to nitrous oxide which is a gaseous end-product. The utility in this process is that nitrate is very soluble as well as inexpensive and use of nitrate mitigates against the high energy requirement for maintaining dissolved oxygen in the process. In fact, nitrate is utilized as an accessory oxidant in some waste water treatment systems (Koch, Gerhard; Siegrist, Hansruedi Verbandsber. - Verb. Schweiz. Abwasser- Gewaesserschutzfachleute (1998), 522 (Optimierungsmassnahmen bei Stark Belasteten Belebungsanlagen), 33-48).

In non-methanotrophic denitrifiers, the microbial process known as denitrification is catalyzed by a series of enzymes which together reductively convert nitrate to gaseous dinitrogen. The steps and intermediates in the process as shown below, together with the enzyme names and gene designations define the scope of the process under consideration.

1.  $\text{NO}_3 \rightarrow \text{NO}_2$  Respiratory nitrate reductase (*Nar* genes).
2.  $\text{NO}_2 \rightarrow \text{NO}$  Respiratory nitrite reductase (*Nir* genes)
3.  $\text{NO} \rightarrow \text{N}_2\text{O}$  Nitric oxide reductase (*Nor* genes)
4.  $\text{N}_2\text{O} \rightarrow \text{N}_2$  Nitrous oxide reductase (*Nos* genes)

Ecologically, the result of these processes is removal of nitrogen from soils (denitrification). However, nitrate can also be viewed as a supplemental or alternative oxidant to oxygen. This is due to the very positive redox potential of the denitrification process.

A second major microbial process is referred to as nitrification and that is comprised of the following set of reactions, enzymes and genes.

1.  $\text{NH}_4 \rightarrow \text{NH}_2\text{OH}$  Ammonia monooxygenase (*amo* genes)
2.  $\text{NH}_2\text{OH} \rightarrow \text{NO}_2$  (Hydroxylamine oxidoreductase )

### 3. $\text{NO}_2 \rightarrow \text{NO}_3$ (Nitrite oxidase)

Nitrification is an oxidative process generating nitrate in soils whereas denitrification is a reductive process depleting nitrate in soils.

- 5 It is well known that obligatory methanotrophic bacteria belong to the group of nitrifying bacteria. This is due to the ability of methane monooxygenase which is found in all obligate methanotrophs to oxygenate ammonia to form hydroxylamine in a reaction identical to that of ammonia monooxygenase and analogous to methane oxygenation to form methanol.
- 10 The hydroxylamine is then further metabolized enzymatically to nitrite. Nitrite oxidation to nitrate can occur enzymatically or spontaneously in air via chemical oxidation. However methanotrophic bacteria have been indirectly associated with denitrification by virtue of their association with denitrifying bacteria such as *Hyphomicrobium* species (Amaral, J.A. Archambault, C. S.R. Richards, R. Knowles 1995. *FEMS Microbiology Ecology* 18 289-298). The respiratory processes described above are distinct from the reduction of nitrate or nitrite for cellular assimilation. The former respiratory process is energy yielding whereas the latter assimilatory process provides nitrogen for incorporation into cellular mass.
- 20 The assimilatory process relies upon pyridine nucleotide linked nitrate or nitrite reductases. These enzymes are widely found in nature including the methanotrophic bacteria. Growth of methanotrophs on nitrate as a sole nitrogen source for biosynthesis is well known in the existing literature (Hanson R.S. A.I. Netrusov, K. Tsuji. 1992. The obligate methanotrophic bacteria *Methylococcus*, *Methylomonas*, and *Methylosinus*. In: The Prokaryotes 2<sup>nd</sup> ed. Ch 18. Pp 2350-2363, A. Balows, H.G. Truper, M. Dworkin, W. Harder, K-H Schleifer eds. Springer Verlag).
- 25

- The functionality of the genes described herein (SEQ ID NO:39-SEQ ID NO:60) lie in the respiratory reduction of nitrate or nitrite to gaseous
- 30  $\text{N}_2\text{O}$ . All genes required to perform this function have been shown to be present in *Methylomonas* 16a both by sequence analysis and physiological reduction of nitrogen containing compounds. Additionally the genes encoding enzymes necessary for the biotransformation of ammonia (nitrification) are also present.

- 35 The advantages to the presence of this denitrification capability in an obligate methanotroph are at least two fold:

1. Nitrate may replace or supplement oxygen as an electron acceptor needed for growth. This can be advantageous for large scale cost-effective cultivation with highly reduced feedstocks that require excessive oxygen demand leading to excessive costs for mass-transfer of gaseous oxygen into solution.
2. Methanotrophic denitrification may be used to remove soluble nitrates from waters or processes where nitrates or other oxygenated nitrogen derivatives are problematic.

Due to the ability of *Methylobacter* 16a to convert ammonia to nitrite combined with the ability to convert nitrite to nitrous oxide demonstrated in the present invention, *Methylobacter* 16a and other methanotrophs which efficiently reduce nitrite can be used as agents to remove ammonia from process waters, waste waters, or natural waters or agricultural effluents for the purpose of clean up and detoxification

Gene Transfer into *Methylobacter* 16a : *Methylobacter* 16a has been shown to accept and express genes from other organisms including *Escherichia coli* and yeast. Several plasmid vectors have been identified which facilitate both gene transfer from a donor organism and expression of the gene in *Methylobacter* 16a. Thus the strain can be genetically engineered.

#### Production of Food and Feed Substrates

It will be appreciated that the present *Methylobacter* 16a strain has the ability to produce, not only proteins, polysaccharides and pigments individually, but may also be engineered to produce a uniquely tailored food or feed product comprising specific quantities and desirable mixtures of these materials. This characteristic of the present strain has significant commercial value.

For example, different livestock animal types may have different nutritional requirements in terms of the relative proportions of protein to carbohydrate. Many carnivorous aquatic fish species, for example, have very high protein requirements. Ruminant livestock, on the other hand, thrive on higher fiber/carbohydrate diets. *Methylobacter* 16a has the capacity to form large amounts of carbohydrate, under certain conditions, in addition to the cellular protein which is always produced. Genes involved in gluconeogenesis (glycogen formation) or glycogen degradation might be altered or regulated such that glycogen content could either be

decreased or increased. Thus the composition of the crude cell mass could be modulated to target high protein feed markets (lower carbohydrate) or alternatively, higher carbohydrate lower protein feed markets. The ability to engineer the composition of the microbe precludes  
5 the need to artificially formulate protein/carbohydrate ratios by exogenous additions.

Carotenoid pigments play a role in terms of providing coloration for many aquatic fish and crustacean species as well as providing antioxidant benefit. (Nelis H.J., De Leenheer 1991. *J. Appl. Bacteriol.* 70:181-191).  
10 *Methylomonas* 16a, unlike many commercially utilized methanotrophs (i.e. *Methylococcus capsulatus*) has a natural carotenoid pigment production pathway which produces high levels of a pink pigment that is similar, but not structurally identical, with such high value carotenoids as astaxanthin. Modification of this pathway by addition of genes involved in the final steps  
15 of astaxanthin synthesis or other high value carotenoids will result in the ability of this strain to produce these carotenoids. In this way *Methylomonas* 16a will be uniquely useful as an animal feed production strain in which the ratios of protein/carbohydrate/pigments may be tailored to suit particular nutritional needs. In this way, *Methylomonas* may be  
20 utilized as a way to deliver higher value components to other sources of plant protein or carbohydrate and thus circumvent the problem of genetic engineering of these plants for the higher value traits.

Methods of manipulating genetic pathways are common and well known in the art. Selected genes in a particular pathway may be  
25 upregulated or down regulated by variety of methods. Additionally, competing pathways in the organism may be eliminated or sublimated by gene disruption and similar techniques.

Once a key genetic pathway has been identified and sequenced specific genes may be upregulated to increase the output of the pathway.  
30 For example, additionally copies of the targeted genes may be introduced into the host cell on multicopy plasmids such as pBR322. Alternatively, the target genes may be modified so as to be under the control of non-native promoters. Where it is desired that a pathway operate at a particular point in a cell cycle or during a fermentation run, regulated or inducible  
35 promoters may be used to replace the native promoter of the target gene. Similarly, in some cases the native or endogenous promoter may be modified to increase gene expression. For example, endogenous

promoters can be altered *in vivo* by mutation, deletion, and/or substitution (see, Kmiec, U.S. Patent 5,565,350; Zarling et al., PCT/US93/03868).

Alternatively it may be necessary to reduce or eliminate the expression of certain genes in the target pathway or in competing pathways that may serve as competing sinks for energy or carbon. Methods of down-regulating genes for this purpose have been explored. Where sequence of the gene to be disrupted is known, one of the most effective methods of gene down regulation is targeted gene disruption where foreign DNA is inserted into a structural gene so as to disrupt transcription. This can be effected by the creation of genetic cassettes comprising the DNA to be inserted (often a genetic marker) flanked by sequence having a high degree of homology to a portion of the gene to be disrupted. Introduction of the cassette into the host cell results in insertion of the foreign DNA into the structural gene via the native DNA replication mechanisms of the cell. (See for example Hamilton et al. (1989) *J. Bacteriol.* 171:4617-4622; Balbas et al. (1993) *Gene* 136:211-213; Gueldener et al. (1996) *Nucleic Acids Res.* 24:2519-2524; and Smith et al. (1996) *Methods Mol. Cell. Biol.* 5:270-277.)

Antisense technology is another method of down regulating genes where the sequence of the target gene is known. To accomplish this, a nucleic acid segment from the desired gene is cloned and operably linked to a promoter such that the anti-sense strand of RNA will be transcribed. This construct is then introduced into the host cell and the antisense strand of RNA is produced. Antisense RNA inhibits gene expression by preventing the accumulation of mRNA which encodes the protein of interest. The person skilled in the art will know that special considerations are associated with the use of antisense technologies in order to reduce expression of particular genes. For example, the proper level of expression of antisense genes may require the use of different chimeric genes utilizing different regulatory elements known to the skilled artisan.

Although targeted gene disruption and antisense technology offer effective means of down regulating genes where the sequence is known, other less specific methodologies have been developed that are not sequence based. For example, cells may be exposed to a UV radiation and then screened for the desired phenotype. Mutagenesis with chemical agents is also effective for generating mutants and commonly used substances include chemicals that affect nonreplicating DNA such as

HNO<sub>2</sub> and NH<sub>2</sub>OH, as well as agents that affect replicating DNA such as acridine dyes, notable for causing frameshift mutations. Specific methods for creating mutants using radiation or chemical agents are well documented in the art. See for example Thomas D. Brock in

5 Biotechnology: A Textbook of Industrial Microbiology, Second Edition (1989) Sinauer Associates, Inc., Sunderland, MA., or Deshpande, Mukund V., *Appl. Biochem. Biotechnol.*, 36, 227, (1992).

Another non-specific method of gene disruption is the use of transposoable elements or transposons. Transposons are genetic

10 elements that insert randomly in DNA but can be latter retrieved on the basis of sequence to determine where the insertion has occurred. Both *in vivo* and *in vitro* transposition methods are known. Both methods involve the use of a transposable element in combination with a transposase enzyme. When the transposable element or transposon, is contacted with

15 a nucleic acid fragment in the presence of the transposase, the transposable element will randomly insert into the nucleic acid fragment. The technique is useful for random mutagenesis and for gene isolation, since the disrupted gene may be identified on the basis of the sequence of the transposable element. Kits for *in vitro* transposition are commercially

20 available ( see for example The Primer Island Transposition Kit, available from Perkin Elmer Applied Biosystems, Branchburg, NJ, based upon the yeast Ty1 element; The Genome Priming System, available from New England Biolabs, Beverly, MA; based upon the bacterial transposon Tn7; and the EZ::TN Transposon Insertion Systems, available from Epicentre

25 Technologies, Madison, WI, based upon the Tn5 bacterial transposable element.

Within the context of the present invention it may be useful to modulate the expression of the identified biosynthetic pathways. For example, it has been noted that the present *Methylobacter* 16a comprises

30 genes encoding both the Entner-Doudoroff and Embden-Meyerhof carbon flux pathways. Because the Embden-Meyerhof pathway is more energy efficient it may be desirable to over-express the genes in this pathway. Additionally, it is likely that the Entner-Doudoroff pathway is a competitive pathway and inhibition of this pathway may lead to increased energy

35 efficiency in the Embden-Meyerhof system. This might be accomplished by selectively using the above described methods of gene down regulation on the sequence encoding the keto-deoxy phosphogluconate aldolase



(SEQ ID NO:9) or any of the other members of the Entner-Doudoroff system and upregulating the gene encoding the fructose bisphosphatase aldolase of the Embden-Meyerhof system (SEQ ID NO:5 OR 7). In this fashion the carbon flux in the present *Methylobacter* 16a may be optimized. Additionally, where the present strain has been engineered to produce specific organic materials such as aromatics for monomer production, optimization of the carbon flux pathway will lead to increased yields of these materials.

In a similar fashion the genes encoding the key enzymes involved in isoprenoid or pigment synthesis may be modulated. For example, the present invention provides a number of genes encoding key enzymes in the terpenoid pathway leading to the production of pigments and smaller isoprenoid compounds. The isolated genes include the *dxs* and *dsr* genes, the *ispA*, *D*, *E*, *F*, and *G* genes, the *pyrG* gene, and *crtN* genes. In particular it may be useful to up-regulate the initial condensation of 3-carbon molecules (pyruvate and C1 aldehyde group, D-glyceraldehyde 3-Phosphate), to yield the 5-carbon compound (D-1-deoxyxylulose-5-phosphate) mediated by the *dxs* gene. Alternatively, if it is desired to produce a specific non-pigmented isoprenoid, it may be desirable to disrupt various genes at the downstream end of the pathway. For example, it may be desirable to use gene disruption or antisense inhibition of the *crtN* gene (known to encode diapophytoene dehydrogenase) if a smaller, upstream terpenoid is the desired product of the pathway.

As has been noted, the present strain has the ability to produce polysaccharides in large amounts. This process is governed by a set of genes including the *ugp* gene, *gumD* and *H* genes, the *epsB*, *M*, and *V* genes and the *waaD* gene. In this pathway it may be of particular importance to up-regulate the *espB* gene involved in polymerization and/or export of the polysaccharide, or the *epsV* gene which controls the transfer of sugar to polysaccharide intermediates.

In this fashion the present strain, or a similar strain may be engineered to produce specific compositions of materials or specific combinations of protein, polysaccharides and pigments for use as a food and feed product.

### EXAMPLES

The present invention is further defined in the following Examples. It should be understood that these Examples, while indicating preferred

embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and  
 5 modifications of the invention to adapt it to various usages and conditions.

#### GENERAL METHODS

Standard recombinant DNA and molecular cloning techniques used in the Examples are well known in the art and are described by Sambrook, J., Fritsch, E. F. and Maniatis, T. *Molecular Cloning: A Laboratory Manual*;  
 10 Cold Spring Harbor Laboratory Press: Cold Spring Harbor, (1989) (Maniatis) and by T. J. Silhavy, M. L. Bannan, and L. W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984) and by Ausubel, F. M. et al., Current Protocols in Molecular Biology, pub. by Greene Publishing Assoc. and Wiley-  
 15 Interscience (1987).

Materials and methods suitable for the maintenance and growth of bacterial cultures are well known in the art. Techniques suitable for use in the following examples may be found as set out in Manual of Methods for General Bacteriology (Phillipp Gerhardt, R. G. E. Murray, Ralph N.  
 20 Costilow, Eugene W. Nester, Willis A. Wood, Noel R. Krieg and G. Briggs Phillips, eds), American Society for Microbiology, Washington, DC. (1994)) or by Thomas D. Brock in Biotechnology: A Textbook of Industrial Microbiology, Second Edition, Sinauer Associates, Inc., Sunderland, MA (1989). All reagents, restriction enzymes and materials used for the growth  
 25 and maintenance of bacterial cells were obtained from Aldrich Chemicals (Milwaukee, WI), DIFCO Laboratories (Detroit, MI), GIBCO/BRL (Gaithersburg, MD), or Sigma Chemical Company (St. Louis, MO) unless otherwise specified.

The meaning of abbreviations is as follows: "h" means hour(s),  
 30 "min" means minute(s), "sec" means second(s), "d" means day(s), "mL" means milliliters, "L" means liters.  
Microbial Cultivation and Preparation of Cell Suspensions, and associated analyses.

*Methylobacter* 16a is typically grown in serum stoppered Wheaton  
 35 bottles using a gas/liquid ratio of at least 8:1 (i.e. 20 mL of Nitrate liquid media) media in a Wheaton bottle (Wheaton Scientific, Wheaton IL) of 160 mL total volume. The standard gas phase for cultivation contained

25% methane in air. These conditions comprise growth conditions and the cells are referred to as growing cells. In all cases the cultures were grown at 30°C with constant shaking in a Lab-Line rotary shaker unless otherwise specified.

- 5 Cells obtained for experimental purposes were allowed to grow to maximum optical density (O.D. 660 ~ 1.0). Harvested cells were obtained by centrifugation in a Sorval RC-5B centrifuge using a SS-34 rotor at 6000 rpm for 20 min. These cell pellets were resuspended in 50 mM HEPES buffer pH 7. These cell suspensions are referred to as washed,  
10 resting cells.

- Microbial growth was assessed in all experiments by measuring the optical density of the culture at 660 nm in an Ultrospec 2000 UV/Vis spectrophotometer (Pharmacia Biotech, Cambridge England) using a 1 cm light path cuvet. Alternatively microbial growth was assessed by harvesting  
15 cells from the culture medium by centrifugation as described above and resuspending the cells in distilled water with a second centrifugation to remove medium salts. The washed cells were then dried at 105°C overnight in a drying oven for dry weight determination.

- Methane concentration was determined as described by Emptage  
20 et al. (1997 *Env. Sci. Technol.* 31:732-734), hereby incorporated by reference.

Nitrate medium for *Methylomonas* 16A

- Nitrate liquid medium, also referred to herein as "defined medium" was comprised of various salts mixed with solution 1 as indicated below or  
25 where specified the nitrate was replaced with 15 mM ammonium chloride.

Solution 1 Composition for 100 fold concentrated stock solution of trace minerals.

	MW	Conc. (mM)	g per L
Nitriloacetic acid	191.1	66.9	12.8
CuCl <sub>2</sub> x 2H <sub>2</sub> O	170.48	0.15	0.0254
FeCl <sub>2</sub> x 4H <sub>2</sub> O	198.81	1.5	0.3
MnCl <sub>2</sub> x 4H <sub>2</sub> O	197.91	0.5	0.1
CoCl <sub>2</sub> x 6H <sub>2</sub> O	237.9	1.31	0.312
ZnCl <sub>2</sub>	136.29	0.73	0.1
H <sub>3</sub> BO <sub>3</sub>	61.83	0.16	0.01
Na <sub>2</sub> MoO <sub>4</sub> x 2H <sub>2</sub> O	241.95	0.04	0.01

NiCl <sub>2</sub> x 6H <sub>2</sub> O	237.7	0.77	0.184
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Mix the gram amounts designated above in 900 mL of H<sub>2</sub>O, adjust to pH=7, and add H<sub>2</sub>O to an end volume of 1 L. Keep refrigerated.

5 Nitrate liquid medium:

	MW	Conc. (mM)	g per L
NaNO <sub>3</sub>	84.99	10	0.85
KH <sub>2</sub> PO <sub>4</sub>	136.09	3.67	0.5
Na <sub>2</sub> SO <sub>4</sub>	142.04	3.52	0.5
MgCl <sub>2</sub> x 6H <sub>2</sub> O	203.3	0.98	0.2
CaCl <sub>2</sub> x 2H <sub>2</sub> O	147.02	0.68	0.1
.1 M HEPES (pH 7)	238.3		50 mL
Solution 1			10 mL

Dissolve in 900 mL H<sub>2</sub>O. Adjust to pH=7, and add H<sub>2</sub>O to give 1 L.

- 10 For agar plates: Add 15 g of agarose in 1 L of medium, autoclave, let cool down to 50°C, mix, and pour plates.

Nitrate and Nitrite Assays

- 15 1 mL samples of cell culture were taken and filtered through a 0.2 micron Acrodisc filter to remove cells. The filtrate from this step contains the nitrite or nitrate to be analyzed. The analysis was performed on a Dionex ion chromatograph 500 system (Dionex, Sunnyvale CA) with an AS3500 autosampler. The column used was a 4 mm Ion-Pac AS11-HC separation column with an AG-AC guard column and an ATC trap column. All columns are provided by Dionex.

- 20 The mobile phase was a potassium hydroxide gradient from 0 to 50 mM potassium hydroxide over a 12 min time interval. Cell temperature was 35°C with a flow rate of 1 mL/min.

Gene Isolation and Characterization

- 25 A number of genes encoding specific identifying enzymes were isolated and sequenced from *Methylobacter* 16a. These include distinguishing genes found in the Entner-Doudoroff carbon flux pathway the Embden-Meyerhof carbon flux pathway, genes encoding a denitrification pathway, genes encoding an isoprenoid synthesis pathway, and genes encoding a pathway for the synthesis of exopolysaccharides. These genes

were sequenced and functionally characterized by comparison of their respective sequences to information in public nucleic acid and protein databases according to the following procedures.

5 Genomic DNA was isolated from *Methylomonas* 16a according to standard protocols. Genomic DNA and library construction were prepared according to published protocols (Fraser et al The Minimal Gene Complement of *Mycoplasma genitalium*; *Science* 270, 1995). A cell pellet was resuspended in a solution containing 100 mM Na-EDTA pH 8.0, 10 mM tris-HCl pH 8.0, 400 mM NaCl, and 50 mM MgCl<sub>2</sub>.

10 Genomic DNA preparation After resuspension, the cells were gently lysed in 10% SDS, and incubated for 30 min at 55°C. After incubation at room temperature, proteinase K was added to 100 µg/mL and incubated at 37°C until the suspension was clear. DNA was extracted twice with tris-equilibrated phenol and twice with chloroform. DNA was  
15 precipitated in 70% ethanol and resuspended in a solution containing 10 mM tris-HCl and 1 mM Na-EDTA (TE) pH 7.5. The DNA solution was treated with a mix of RNAases, then extracted twice with tris-equilibrated phenol and twice with chloroform. This was followed by precipitation in ethanol and resuspension in TE.

20 Library construction 200 to 500 µg of chromosomal DNA was resuspended in a solution of 300 mM sodium acetate, 10 mM tris-HCl, 1 mM Na-EDTA, and 30% glycerol, and sheared at 12 psi for 60 sec in an Aeromist Downdraft Nebulizer chamber (IBI Medical products, Chicago, IL). The DNA was precipitated, resuspended and treated with Bal31  
25 nuclease. After size fractionation, a fraction (2.0 kb, or 5.0 kb) was excised, cleaned and a two-step ligation procedure was used to produce a high titer library with greater than 99% single inserts.

Sequencing A shotgun sequencing strategy approach was adopted for the sequencing of the whole microbial genome (Fleischmann, Robert  
30 et al Whole-Genome Random sequencing and assembly of *Haemophilus influenzae* Rd *Science* , 269: 1995).

Sequence was generated on an ABI Automatic sequencer using dye terminator technology (US 5366860; EP 272007) using a combination of vector and insert-specific primers. Sequence editing was performed in  
35 either DNASTar (DNA Star Inc.) or the Wisconsin GCG program (Wisconsin Package Version 9.0, Genetics Computer Group (GCG),

Madison, WI) and the CONSED package (version 7.0). All sequences represent coverage at least two times in both directions.

#### Microarray of gene expression

##### Amplification of DNA regions for the construction of DNA

5 microarray: Specific primer pairs were used to amplify each protein specifying ORF of *Methylobacter* sp. strain 16a. Genomic DNA (10-30 ng) was used as the template. The PCR reactions were performed in the presence of HotStart Taq™ DNA polymerase (Qiagen, Valencia, CA) and the dNTPs (Gibco BRL Life Science Technologies, Gaithersburg, MD)  
10 ). Thirty-five cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec and polymerization at 72°C for 2 min were conducted. The quality of PCR reactions was checked with electrophoresis in a 1% agarose gel. The DNA samples were purified by the high-throughput PCR purification kit from Qiagen.

15 Arraying amplified ORFs. Before arraying, an equal volume of DMSO (10 µL) and DNA (10 µL) sample was mixed in 384-well microtiter plates. A generation II DNA spotter (Molecular Dynamics, Sunnyvale, CA) was used to array the samples onto coated glass slides (Telechem, Sunnyvale, CA). Each PCR product was arrayed in duplicate on each  
20 slide. After cross-linking by UV light, the slides were stored under vacuum in a desiccator at room temperature.

RNA isolation: *Methylobacter* 16a was cultured in a defined medium with ammonium or nitrate (10 mM) as nitrogen source under 25% methane in air. Samples of the minimal medium culture were harvested  
25 when the O.D. reaches 0.3 at A<sub>600</sub> (exponential phase). Cell cultures were harvested quickly and ruptured in RLT buffer [Qiagen RNeasy Mini Kit, Valencia, CA] with a beads-beater (Bio101, Vista, CA). Debris was pelleted by centrifugation for 3 min at 14,000 x g at 4°C. RNA isolation was completed using the protocol supplied with this kit. After on-column  
30 DNAase treatment, the RNA product was eluted with 50-100 µL RNAase-free. RNA preparations were stored frozen at either -20 or -80°C.

Synthesis of fluorescent cDNA from total RNA. RNA samples (7 to 15 µg) and random hexamer primers (6 µg; Gibco BRL Life Science Technologies) were diluted with RNAase-free water to a volume of 25 µL.  
35 The sample was denatured at 70°C for 10 min and then chilled on ice for 30 seconds. After adding 14 µL of labeling mixture, the annealing was accomplished by incubation at room temperature for 10 min. The labeling

mixture contained 8  $\mu$ L of 5x enzyme buffer, 4  $\mu$ L DTT (0.1M), and 2  $\mu$ L of 20x dye mixture. The dye mixture consisted of 2 mM of each dATP, dGTP, and dTTP, 1 mM dCTP, and 1 mM of Cy3-dCTP or Cy5-dCTP. After adding 1 to 1.5  $\mu$ L of SuperScript II reverse transcriptase

- 5 (200 units/mL, Life Technologies Inc., Gaithersburg, MD), cDNA synthesis was allowed to proceed at 42°C for 2 hr. The RNA was removed by adding 2  $\mu$ L NaOH (2.5 N) to the reaction. After 10 min of incubation at 37°C, the pH was adjusted with 10  $\mu$ L of HEPES (2M). The labeled cDNA was then purified with a PCR purification kit (Qiagen, Valencia, CA).
- 10 Labeling efficiency was monitored using either  $A_{550}$  for Cy3 incorporation, or  $A_{650}$  for Cy5.

- Fluorescent labeling of genomic DNA. Genomic DNA was nebulized to approximately 2 kb pair fragments. Genomic DNA (0.5 to 1  $\mu$ g) was mixed with 6  $\mu$ g of random hexamers primers (Gibco BRL Life
- 15 Science Technologies) in 15  $\mu$ L of water. The mix was denatured by put at boiling water for 5 minutes. Then anneal on ice for 30 sec before put at room temperature. Then 2  $\mu$ L 5x Buffer 2 (Gibco BRL) and 2  $\mu$ L dye mixture were added. The component of dye mixture and the labeling procedure are the same as described above for RNA labeling, except that
- 20 the Klenow fragment of DNA polymerase I (5  $\mu$ g/ $\mu$ L, Gibco BRL Life Science Technologies) was used as the enzyme. After incubation 37 °C for 2 hr, the labeled DNA probe was purified using a PCR purification kit (Qiagen, Valencia, CA).

- Hybridization and washing. Slides were first incubated with
- 25 prehybridization solution containing 3.5xSSC (BRL, Life Technologies Inc., Gaithersburg, MD), 0.1% SDS (BRL, Life Technologies Inc., Gaithersburg, MD), 1% bovine serum albumin (BSA, Fraction V, Sigma, St. Louis, MO). After prehybridization, hybridization solutions (Molecular Dynamics) containing labeled probes was added to slides and covered with cover
- 30 slips. Slides were placed in a humidified chamber in a 42°C incubator. After overnight hybridization, slides were initially washed for 5 min at room temperature with a washing solution containing 1xSSC, 0.1% SDS and 0.1xSSC, 0.1% SDS. Slides were then washed at 65°C for 10 min with the same solution for three times. After washing, the slides were dried
- 35 with a stream of nitrogen gas.

Data Collection and Analysis. The signal generated from each slide was quantified with a laser scanner (Molecular Dynamics, Sunnyvale, CA).

The images were analyzed with ArrayVision 4.0 software (Imaging Research, Inc., Ontario, Canada). The raw fluorescent intensity for each spot was adjusted by subtracting the background. These readings were exported to a spreadsheet for further analysis.

5        Table 1 is a description of the genes discovered and annotated for *Methylobacter* 16a. The table shows sequence % similarities, % identities, and expectation values for key genes of central carbon metabolism, denitrification, exopolysaccharide synthesis, and isoprenoid biosynthesis.

10        Table 1 illustrates the relationship of these sequences to known sequences in the art. All sequences were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul, S. F., et al., (1993) *J. Mol. Biol.* 215:403-410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The sequences were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish, W. and States, D. J. (1993) *Nature Genetics* 3:266-272) provided by the NCBI. All comparisons were done using either the BLASTNnr or BLASTXnr algorithm. The results of the BLAST comparison is given below in Table 1 which summarize the sequences to which they have the most similarity. Table 1 displays data based on the BLASTXnr algorithm with values reported in expect values. The Expect value estimates the statistical significance of the match, specifying the number of matches, with a given score, that are expected in a search of a database of this size absolutely by chance.

20  
25  
30



**Table 1**  
**Genes Characterized From *Methylobacter* 16a**

Gene Name	Similarity Identified	SEQ ID	SEQ ID Peptide	% Identity a	% Similarity b	E-value c	Citation
Phosphoglucose mutase	Phosphoglucose mutase (Glucose Phosphomutase) >> gil3241933 gilAA D03475.1	1	2	65%	85%	1.7e-140	Lepek et al., Direct Submission  gilAA D03475.1
Glucose 6 phosphate isomerase	Glucose 6 phosphate isomerase gil396360 gil AAC43119.1	3	4	64%	81%	1.6e-136	Blattner et al., Nucleic Acids Res. 21 (23), 5408-5417 (1993)
Phosphofructose kinase pyrophosphate dependent	Phosphofructose kinase pyrophosphate dependent gil150931 gil AAA25675.1  (M67447)	5	6	63%	83%	1.7e-97	Lador et al., J. Biol. Chem. 266, 16550-16555 (1991)
6-Phosphoglucose dehydratase	6-Phosphoglucose dehydratase gil4210902 gil AAD12045.1  (AF045609)	7	8	60%	85%	1.6e-141	Willis et al., J. Bacteriol. 181 (14), 4176-4184 (1999)

Gene Name	Similarity Identified	SEQ ID	SEQ ID Peptide	% Identity a	% Similarity b	E-value c	Citation
Glucose 6 phosphate 1 dehydrogenase	Glucose 6 phosphate 1 dehydrogenase gi 397854 emb CAA52858.1  (X74866)	9	10	58%	85%	9.4e-123	Hugouvieux-Cotte-Pattat N, TITLE Direct Submission, gi 397854 emb CAA52858.1  (X74866)
TAL	Transaldolase	11	12	78%	90%	2.7e-92	Plant Mol. Biol. 30 (1), 213-218 (1996)
MIPB	Transaldolase	13	14	50%	79%	1e-23	Blatner F.R. et al Science 277:1453-1474(1997).
FBA or FDA	Fructose biphosphate aldolase	15	16	76%	92%	4.1e-111	Alefounder P.R. et al. Mol. Microbiol. 3:723-732(1989).
FBA or FDA	Fructose biphosphate aldolase	17	18	40%	70%	2.3e-39	van den Bergh E.R. et al.; J. Bacteriol. 178:888-893 (1996).
KHG/KDPG	(AL352972) KHG/KDPG aldolase Streptomyces coelicolor	19	20	59%	72%	1e-64	Redenbach et al., Mol. Microbiol. 21 (1), 77-96 (1996)
ugp	ugp (Xanthomonas campestris)	21	22	58%	82%	3.2 e-60	Wei et al., Biochem. Biophys. Res. Commun. 226 (3), 607-612 (1996)

Gene Name	Similarity Identified	SEQ ID	SEQ ID Peptide	% Identity a	% Similarity b	E-value c	Citation
<i>gumD</i>	<i>gumD</i> ( <i>Xanthomonas campestris</i> )	23	24	36%	69%	2.5 e-52	Chou, F. L., et al., <i>Biochem. Biophys. Res. Commun.</i> 233 (1), 265-269 (1997)
<i>wza</i>	<i>wza</i> ( <i>Escherichia coli</i> )	25	26	36%	69%	5.8 e-39	Blattner, F. R. et al., <i>Science</i> 277 (5331), 1453-1474 (1997)
<i>epsB</i>	<i>epsB</i> ( <i>Pseudomonas solanacearum</i> )	27	28	35%	67%	2 e-74	Huang, J. and Schell, M., <i>Mol. Microbiol.</i> 16 (5), 977-989 (1995)
<i>epsM</i>	<i>epsM</i> ( <i>Streptococcus thermophilus</i> )	30	20	23%	55%	1.3 e-05	Stingele, F. et al., <i>J. Bacteriol.</i> 178 (6), 1680-1690 (1996)
<i>waaE</i>	<i>waaE</i> ( <i>Serratia marcescens</i> )	31	32	28%	55%	8.6 e-09	Pique, N. et al., Unpublished Genbank number: AAC44433
<i>epsV</i>	<i>epsV</i> ( <i>Streptococcus thermophilus</i> )	33	34	21%	56%	2.3 e-05	Bourgoin, F. et al., <i>Plasmid</i> 40 (1), 44-49 (1998)
<i>gumH</i>	<i>gumH</i> ( <i>Rhizobium meliloti</i> )	35	36	26%	55%	0.00088	Becker, A. et al., <i>Mol. Microbiol.</i> 16 (2), 191-203 (1995)

Gene Name	Similarity Identified	SEQ ID	SEQ ID Peptide	% Identity a	% Similarity b	E-value c	Citation
glycosyl transferase	Glycosyltransferase ( <i>Actinobacillus</i> <i>actinomycetemcomitans</i> )	37	38	51%	80%	1.7 e-62	Nakano, Y, Biochem. Biophys. Acta 1442:409-414 (1998)
nirF	NirF protein ( <i>Pseudomonas</i> )	39	40	59%	85%	1.3e-92	Palmedo et al., Eur. J. Biochem. 232 (3), 737-746 (1995)
nirD	NirD protein ( <i>Pseudomonas</i> )	41	42	49%	76%	1.7e-22	Palmedo et al., Eur. J. Biochem. 232 (3), 737-746 (1995)
nirL	NirL protein ( <i>Pseudomonas</i> )	43	44	49%	73%	6.4e-28	Palmedo et al., Eur. J. Biochem. 232 (3), 737-746 (1995)
nirG	NirG protein ( <i>Pseudomonas</i> )	45	46	49%	80%	1.6e-25	Kawasaki et al., J. Bacteriol. 179 (1), 235-242 (1997)
nirH	NirH protein ( <i>Pseudomonas</i> )	47	48	59%	78%	9.9e-33	Kawasaki et al., J. Bacteriol. 179 (1), 235-242 (1997)
nirJ	NirJ protein ( <i>Pseudomonas</i> )	49	50	56%	81%	5.1e-88	Kawasaki et al., J. Bacteriol. 179 (1), 235-242 (1997)
nasA	Nitrate reductase <i>Klebsiella</i>	51	52	51%	74%	9.2e-123	LIN J.T., GOLDMAN B.S., STEWART V., J. Bacteriol. 175:2370-2378(1993).

Gene Name	Similarity Identified	SEQ ID	SEQ ID Peptide	% Identity a	% Similarity b	E-value c	Citation
norC	Nitric-oxide reductase subunit C ( <i>Pseudomonas</i> )	53	54	32%	70%	1e-08	Zumft et al., Eur. J. Biochem. 219:481-490(1994).
norB	Nitric-oxide reductase subunit B ( <i>Pseudomonas</i> )	55	56	39%	70%	3.5e-64	Zumft et al., Eur. J. Biochem. 219:481-490(1994).
norZ	Cytochrome B subunit of nitric oxide reductase ( <i>Alcaligenes</i> )	57	58	39%	69%	1.7e-100	Cramm, R., Siddiqui, R.A. and Friedrich, B. J. Bacteriol. 179 (21), 6769-6777 (1997).
norS	Nitrite reductase (cytochrome cd1) ( <i>Pseudomonas</i> )	59	60	28%	59%	2.1e-25	Glockner, A.B. and Zumft, W.G. Biochim. Biophys. Acta 1277 (1-2), 6-12 (1996).
dxs	1-deoxyxylulos e-5-phosphate synthase	61	62	60%	86%	5.7e-149	Lois, L.M., et al., Proc. Natl. Acad. Sci. U.S.A. 95 (5), 2105-2110 (1998)

Gene Name	Similarity Identified	SEQ ID	SEQ ID Peptide	% Identity a	% Similarity b	E-value c	Citation
<i>dxr</i>	1-deoxy-d-xylulose 5-phosphate reductoisomerase	63	64	55%	78%	3.3e-74	Takahashi S et al., Proc. Natl. Acad. Sci. U.S.A. 95:9879-9884(1998).
<i>ygbB/ispF</i>	2C-methyl-d-erythritol 2,4-cyclodiphosphate synthase	65	66	69%	84%	1.6e-36	Herz S, et al., Proc Natl Acad Sci U S A 2000 Mar 14;97(6):2486-90
<i>ygbP/ispD</i>	2C-methyl-d-erythritol cytidyltransferase	67	68	52%	74%	7.7e-36	Rohdich F, et al., Proc Natl Acad Sci U S A 1999 Oct 12;96(21):11758-63
<i>pyrG</i>	CTP synthase	69	70	67%	89%	2.4e-141	Weng M., J. et al., Biol. Chem. 261:5568-5574(1986).
<i>ispA</i>	Geranyltransferase (also farnesyl-diphosphate synthase)	71	72	56%	78%	7.8e-56	Ohno, C et al., Plant Mol. Biol. 40 (2), 307-321 (1999)
<i>ychB/ispE</i>	4-diphosphocytidylyl-2-C-methylerythritol kinase	73	74	50%	72%	8.8e-49	Lutigen H, Proc Natl Acad Sci U S A. 2000 Feb 1;97(3):1062-7.

Gene Name	Similarity Identified	SEQ ID	SEQ ID Peptide	% Identity a	% Similarity b	E-value c	Citation
<i>crtN1</i>	diapophytoene dehydrogenase Crtn— copy 1	75	76	34%	72%	4e-66	Xiong, J. Proc. Natl. Acad. Sci. U.S.A. 95 (25), 14851-14856 (1998)
<i>crtN2</i>	Diapophytoene dehydrogenase Crtn— copy 2	77	78	49%	78%	1.3e-76	Wieland, K.P. and Goetz, F. Unpublished
Particulate methane monooxygenase	probable methane monooxygenase 45k chain - Methylococcus capsulatus B57266 GI:2120829	79	80	71%	85%	0.0	Semrau et al., J. Bacteriol. 177 (11), 3071-3079 (1995)

a% Identity is defined as percentage of amino acids that are identical between the two proteins.

b% Similarity is defined as percentage of amino acids that are identical or conserved between the two proteins.

c Expect value. The Expect value estimates the statistical significance of the match, specifying the number of matches, with a given score, that are expected in a search of a database of this size absolutely by chance

### EXAMPLE 1

#### ISOLATION OF METHYLOMONAS 16A

The original environmental sample containing the isolate was obtained from pond sediment. The pond sediment was inoculated directly  
5 into defined medium with ammonium as nitrogen source under 25% methane in air. Methane was the sole source of carbon and energy. Growth was followed until the optical density at 660 nm was stable whereupon the culture was transferred to fresh medium such that a 1:100 dilution was achieved. After 3 successive transfers with methane as sole  
10 carbon and energy source the culture was plated onto growth agar with ammonium as nitrogen source and incubated under 25% methane in air. Many methanotrophic bacterial species were isolated in this manner. However, *Methylomonas* 16a was selected as the organism to study due to the rapid growth of colonies, large colony size, ability to grow on minimal  
15 media, and pink pigmentation indicative of an active biosynthetic pathway for carotenoids.

### EXAMPLE 2

#### RAPID GROWTH ON METHANE IN MINIMAL MEDIUM

*Methylomonas* 16a grows on the defined medium comprised of only  
20 minimal salts, a culture headspace comprised of methane in air. Methane concentrations for growth but typically are 5-50% by volume of the culture headspace. No organic additions such as yeast extract or vitamins are required to achieve growth shown in Figure 1. Figure 1 shows the growth of 16a compared to the growth of *Methylococcus capsulatus* under  
25 identical growth conditions. i.e. minimal medium with 25% methane in air as substrate. The data indicates *Methylomonas* 16a doubles every 2-2.5 h whereas *Methylococcus capsulatus* doubles every 3.5 h with methane as substrate. With methanol as substrate doubling times on methanol are  
30 2.5-3 for *Methylomonas* 16a and 4.5-5 for *Methylococcus capsulatus*. Cell densities are also significantly higher for *Methylomonas* 16a growing on methane. *Methylococcus capsulatus* is a widely utilized methanotroph for experimental and commercial purposes.

### EXAMPLE 3

#### METHANOL TOLERANCE

35 *Methylomonas* 16a was grown on defined medium with nitrate as sole nitrogen source and methanol as sole carbon source. Growth was monitored over a 36 hr period which was typically sufficient for attaining maximum optical density or turbidity of the culture. Figure 2 clearly shows



that maximum growth or turbidity is attained within 36 hours at methanol concentrations up to 600 mM. However no growth was observed at 800 mM. Therefore the strain is shown to grow on 2.4% (vol/vol) of methanol.

#### EXAMPLE 4

##### PROPERTIES AND CLASSIFICATION OF METHYLOMONAS 16A

Table 2 shows the various properties of *Methylomonas* 16a. The criteria listed in Table 2 are those typically used to determine whether the strain is arbitrarily considered Type I, Type II or Type X based on physical and enzymatic properties. This table was developed from both direct enzymatic assay for enzymes as well as genomic data showing the presence of genes and gene pathways. This categorization is functionally based and indicates that the strain utilizes the most energetically efficient pathway for carbon incorporation which is the ribulose monophosphate or "RuMP" pathway. Genomic data clearly shows the presence of key enzymes in the RuMP pathway. Internal membrane structure are also indicative of a Type I physiology. Unique to the present strain is the finding of nitrogen fixation genes in *Methylomonas* 16a. The strain is shown to grow in the absence of yeast extract or vitamins. Nitrate, ammonium ion or dinitrogen can satisfy the nitrogen requirement for biosynthesis. This functional data is in complete agreement with the 16sRNA homologies as compared with other *Methylomonas* strains. 16sRNA comparisons of the 16a strain (SEQ ID NO:81) with other *Methylomonas* sp. revealed that *Methylomonas* 16a has 96% identity with the 16sRNA of *Methylomonas* sp. (strain:KSPIII) [Hanada, S et al., *J. Ferment. Bioeng.* 86, 539-544 (1998)] and with *Methylomonas* sp. (strain LW13), [Costello, A.M. and Lidstrom, M.E. *Appl. Environ. Microbiol.* 65 (11), 5066-5074 (1999)]. Thus *Methylomonas* 16a is correctly classified as a Type I, RuMP utilizing, *Methylomonas* species.

Table 2

Characteristic	Type I	<i>Methylobacterium</i> 16a	Type X	Type II
%GC	Incomplete	Incomplete	Incomplete	Complete
Ribmp Cycle	Incomplete	Incomplete	Incomplete	Complete
RuBP Carboxylase	-	-	+	+
Temp. Range	<45	<42	<45	<40
Nitrogenase	-	+	+	+
G6P dehydrogenase NADP	+	+	+	-
Isocitrate dehydrogenase NAD/NADP	+	+	-	-
Yeast Extract	-	-	-	-
Vitamins	-	-	-	-
Pigmentation	Variable	+	Variable	Variable
Nitrate assimilation	+	+	+	+

#### Method of enzymatic assay

- 5 Nitrogenase was not assayed but is considered positive if the gene is present on the basis of genome sequence analysis.

- 10 Glucose 6 phosphate dehydrogenase: One mL of reaction mixture contains 100  $\mu$ L of 10 mM NADP, 100  $\mu$ L of 10 mM glucose, 700  $\mu$ L of 100 mM HEPES pH 7 buffer and up to 100  $\mu$ L of enzyme extract. The enzyme activity was measured by monitoring NADP reduction to NADPH at 340 nm using spectrophotometer.

- 15 Isocitrate dehydrogenase: One mL of reaction mixture contains 100  $\mu$ L of 10 mM sodium isocitrate, 100  $\mu$ L of 10 mM NADP, 700  $\mu$ L of 100 mM pH 7 HEPES buffer up to 100  $\mu$ L of enzyme extract. The enzyme activity was measured by monitoring NADPH formation at 340 nm.

- 20 Nitrate assimilation is based on the ability of the strain to grow on nitrate as sole nitrogen source.

The results of the enzyme assay are shown in Table 2.

EXAMPLE 5  
COMPARISON OF GENE EXPRESSION LEVELS IN THE ENTNER  
DOUDEROFF PATHWAY AS COMPARED WITH THE EMBDEN  
MEYERHOF PATHWAY

5           Example 5 presents microarray evidence for the use of the Embden-Meyerhof pathway in the 16a strain.

          Figure 3 shows the relative levels of expression of genes for the Entner-Douderoﬀ pathway and the Embden-Meyerhof pathway. The relative transcriptional activity of each gene was estimated with DNA  
10   microarray as described previously (Wei, *et al.*, 2001. *Journal of Bacteriology*. 183:545-556).

          Specifically, a single DNA microarray containing 4000 ORFs (open reading frames) of *Methylobacter* sp. strain 16a was hybridized with probes generated from genomic DNA and total RNA. The genomic DNA of  
15   16a was labeled with Klenow fragment of DNA polymerase and fluorescent dye Cy-5, while the total RNA was labeled with reverse transcriptase and Cy-3. After hybridization, the signal intensities of both Cy-3 and Cy-5 for each spot in the array were quantified. The intensity ratio of Cy-3 and Cy-5 was then used to calculate the fraction of each transcript (in percentage)  
20   with the following formula: (gene ratio/sum of all ratio) x 100. The value obtained reflects the relative abundance of mRNA of an individual gene. Accordingly, transcriptional activity of all the genes represented by the array can be ranked based on its relative mRNA abundance in a descending order. For example, mRNA abundance for the methane  
25   monooxygenase was ranked #1 because its genes had the highest transcriptional activity when the organism was grown with methane as the carbon source (Figure 3).

          The genes considered "diagnostic" for Entner-Douderoﬀ are the 6 phosphogluconate dehydratase and the 2 keto-3-deoxy-6-  
30   phosphogluconate aldolase. Phosphofructokinase and fructose biphosphate aldolase are "diagnostic" of the Embden-Meyerhof sequence. Numbers in Figure 3 next to each step indicate the relative expression level of that enzyme. For example the most highly expressed enzyme in the cell is the methane monooxygenase (ranked #1). The next most highly  
35   expressed is the methanol dehydrogenase (ranked #2). Messenger RNA transcripts of Phosphofructokinase (ranked #232) and fructose biphosphate aldolase (ranked #65) were in higher abundance than those for glucose 6 phosphate dehydrogenase (ranked #717), 6

phosphogluconate dehydratase (ranked #763) or the 2-keto-3-deoxy-6-gluconate aldolase. The data suggests that the Embden-Meyerhof pathway enzymes are more strongly expressed than the Entner-Doudoroff pathway enzymes. This result is surprising and counter to existing beliefs on the central metabolism of methanotrophic bacteria (Reference book pages in. The physiology and biochemistry of aerobic methanol-utilizing gram-negative and gram-positive bacteria In: Methane and Methanol Utilizers, Biotechnology Handbooks 5. 1992. Eds: Colin Murrell, Howard Dalton. Pp 149-157.

#### EXAMPLE 6

#### DIRECT ENZYMATIC EVIDENCE FOR A PYROPHOSPHATE-LINKED PHOSPHOFRUCTOKINASE

Example 6 shows the evidence for the presence of a pyrophosphate-linked phosphofructokinase enzyme in the current strain which would confirm the functionality of the Embden-Meyerhof pathway in the present strain.

Phosphofructokinase activity was shown to be present in *Methylobacter* 16a by using the coupled enzyme assay described below. Assay conditions are given in Table 3 below. This assay was further used to assay the activity in a number of other Methanotrophic bacteria as shown below in Table 4. The data in Table 4 show known ATCC strains tested for phosphofructokinase activity with ATP or pyrophosphate as phosphoryl donor. These organisms were classified as either Type I or Type X ribulose monophosphate-utilizing strains or Type II serine utilizer.

#### Coupled Assay Reactions

Phosphofructokinase reaction is measured by a coupled enzyme assay. Phosphofructokinase reaction is coupled with fructose 1,6, biphosphate aldolase followed by triosephosphate isomerase. The enzyme activity is measured by the disappearance of NADH.

Specifically, the enzyme phosphofructokinase catalyzes the key reaction converting Fructose 6 phosphate and pyrophosphate to Fructose 1,6 bisphosphate and orthophosphate.

Fructose-1,6-bisphosphate is cleaved to 3-phosphoglyceraldehyde and dihydroxyacetonephosphate by fructose 1,6-bisphosphate aldolase.

Dihydroxyacetonephosphate is isomerized to 3-phosphoglyceraldehyde by triosephosphate isomerase.

Glycerol phosphate dehydrogenase plus NADH and 3-phosphoglyceraldehyde yields the alcohol glycerol-3-phosphate and NAD.

Disappearance of NADH is monitored at 340 nm using spectrophotometer (UltraSpec 4000, Pharmacia Biotech).

Table 3  
Assay Protocol

5

Reagent	Stock solution (mM)	Volume ( $\mu$ l) per 1mL total reaction volume	Final assay concentration (mM)
Tris-HCl pH 7.5	1000	100	100
MgCl <sub>2</sub> · 2 H <sub>2</sub> O	100	35	3.5
Na <sub>4</sub> P <sub>2</sub> O <sub>7</sub> · 10H <sub>2</sub> O or ATP	100	20	2
Fructose-6-phosphate	100	20	2
NADH	50	6	0.3
Fructose biphosphate aldolase	100 ( units/mL)	20	2 (units)
Triose phosphate isomerase/glyceral phosphate dehydrogenase	(7.2 units/ $\mu$ l) (0.5 units/ $\mu$ l)	3.69	27 units 1.8 units
KCl	1000	50	50
H <sub>2</sub> O		adjust to 1mL	
Crude extract		0-50	

**Table 4**  
**Comparison Of Pyrophosphate Linked And ATP Linked**  
**Phosphofructokinase Activity In Different Methanotrophic Bacteria**

Strain	Type	Assimilation Pathway	ATP-PFK umol NADH/ min/mg	Ppi-PFK umol NADH/ min/mg
Methylomonas 16a ATCC PTA 2402	I	Ribulose monophosphat e	0	2.8
Methylomonas agile ATCC 35068	I	Ribulose monophosphat e	0.01	3.5
Methylobacter Whittenbury ATCC 51738	I	Ribulose monophosphat e	0.01	0.025
Methylomonas clara ATCC 31226	I	Ribulose monophosphat e	0	0.3
Methylomicrobium albus ATCC 33003	I	Ribulose monophosphat e	0.02	3.6
Methylococcus capsulatus ATCC 19069	X	Ribulose monophosphat e	0.01	0.04
Methylosinus sporum ATCC 35069	II	Serine	0.07	0.4

5

Several conclusions may be drawn from the data presented above. First, it is clear that ATP (which is the typical phosphoryl donor for phosphofructokinase) is essentially ineffective in the phosphofructokinase reaction in methanotrophic bacteria. Only inorganic pyrophosphate was found to support the reaction in all methanotrophs tested. Secondly not all methanotrophs contain this activity. The activity was essentially absent in *Methylobacter whittenbury* and in *Methylococcus capsulatus*. Intermediate levels of activity were found in *Methylomonas clara* and *Methylosinus sporium*. These data show that many methanotrophic bacteria may contain a hitherto unreported phosphofructokinase activity. It may be inferred from this that methanotrophs containing this activity have an active Embden-Meyerhof pathway.

10

15

EXAMPLE 7GROWTH YIELD AND CARBON CONVERSION BY METHYLOMONAS16A

Growth yield and carbon conversion efficiency were compared for  
 5 *Methylomonas* 16a and *Methylococcus capsulatus*. These strains were  
 chosen because 16a contains high levels of phosphofructokinase and *M.*  
*capsulatus* is essentially devoid of the enzyme activity. It was  
 contemplated that if *Methylomonas* 16a could utilize the more energetically  
 favorable Embden-Meyerhof pathway and *Methylococcus capsulatus* could  
 10 only use the Entner-Doudoroff pathway the superior energetics of the  
 present *Methylomonas* 16a strain would be reflected in cellular yields and  
 carbon conversion efficiency. This difference in energetic efficiency would  
 only be apparent under energy-limiting conditions. These conditions were  
 achieved in this experiment by limiting the amount of oxygen in each  
 15 culture to only 10% (vol/vol) instead of 20% (growth conditions employed in  
 Figure 1 and Table 9). Under these oxygen limiting conditions the strain  
 that produces the most energy from aerobic respiration on methane will  
 produce more cell mass.

Cells were grown as 200 mL cultures 500 mL serum-stoppered  
 20 Wheaton bottles. The headspace in the bottles was adjusted to 25%  
 methane and 10% oxygen. The defined medium formulation is the same in  
 both cases.

Table 5

25 Yield Of *Methylomonas* 16a Cells Versus *Methylococcus Capsulatus* Cells  
Under Oxygen Limitation.

Strain	$Y_{CH_4}$ g dry wt/mol	G dry wt/g $CH_4$	<u>Carbon Conversion</u> <u>Efficiency</u> (CCE)%
<i>Methylomonas</i> 16a	16.7 +/- 0.5	1.04	64
<i>Methylococcus</i> <i>capsulatus</i>	10.3 +/- 0.3	0.64	40

Yield determination: Yield was measured by growing triplicate  
 30 cultures in 500 mL bottles on defined medium with ammonium as nitrogen  
 source under oxygen limitation. This was done by using 300 mL of culture  
 with a 300 mL headspace of 25% methane and 10% oxygen the balance

being nitrogen. At the end of growth (i.e. stationary phase) residual methane in the headspace was determined by gas chromatography. The cells were collected by centrifugation washed with distilled water and dried overnight in a drying oven before being weighed.

- 5 Carbon conversion efficiency is a measure of how much carbon is assimilated into cell mass. It is calculated assuming a biomass composition of  $\text{CH}_2\text{O}_{0.5}\text{N}_{0.25}$ :

*Methylomonas 16a* :  $16 \text{ g/mol methane} \times (1 \text{ g dry wt/g methane}) / 25 \text{ g/mol biomass}$

- 10 *M. capsulatus*  $16 \text{ g/mol methane} \times (0.64 \text{ g dry wt/g methane}) / 25 \text{ g/mol biomass}$

These data (in Table 5) show that *Methylomonas 16a* produced significantly more cell mass than did the *Methylococcus capsulatus* strain under growth conditions that were identical except for the temperature.

- 15 *Methylococcus capsulatus* grows optimally at 45°C whereas *Methylomonas* is grown at 33°C. It may be inferred from the data that the presence of the more energy-yielding Embden-Meyerhof pathway confers a growth advantage to *Methylomonas 16a*.

- Table 6 presents the theoretical calculations showing ATP yield as a function of carbon assimilation pathway with the carbon output being normalized to pyruvate in all cases. (The physiology and biochemistry of aerobic methanol-utilizing gram-negative and gram-positive bacteria In: Methane and Methanol Utilizers, Biotechnology Handbooks 5. 1992. Eds: Colin Murrell, Howard Dalton. Pp. 149-157). Table 6 shows the amount of ATP that is produced or consumed for every three molecules of carbon (as formaldehyde or carbon dioxide) for serine cycle, xylulose monophosphate cycle and ribulose monophosphate cycle pathways. The latter pathway, as discussed is typically thought to exist as the 2-keto-3deoxy-6-phosphogluconate /transaldolase (KDPGA/TA) variant. These data shows that in fact the fructose biphosphate aldolase/transaldolase (FBPA/TA) variant is likely to exist in the methanotrophs. The energetic repercussion of this is the net production of an additional 1 ATP for methanotrophs if they possess an ATP linked phosphofructokinase and an additional 2 ATPs for the pyrophosphate-linked enzyme. It is therefore expected that *Methylomonas 16a* derives an additional 2 ATP per 3 carbons assimilated and that this may explain the greater yield and carbon efficiency of the strain versus *Methylococcus capsulatus*.



Table 6  
Energetics of Methanotrophic bacteria utilizing different carbon  
assimilation mechanisms

Organism	Cycle	C1 unit fixed	Product	Variant	ATP	NADPH
Bacteria	RuMP	3CH <sub>2</sub> O	Pyruvate	FBPA/TA	+1	+1
Methylomonas	RuMP/Serine	3CH <sub>2</sub> O	Pyruvate	FBPA/TA	+1(+2*)	+1
Bacteria	RuMP	3CH <sub>2</sub> O	Pyruvate	KDPGA/TA	0	+1
Methylococcus	RuMP/RuBP	3CH <sub>2</sub> O	Pyruvate	KDPGA/TA	0	+1

5     \* Based on PPI dependent phosphofructokinase

### EXAMPLE 8 NITRATE/NITRITE SPARES OXYGEN

Figure 4 shows oxygen uptake by a cell suspension of

10     *Methylomonas* 16a, in relative detector units, using an Orion oxygen probe (Orion, UK) to detect oxygen consumption. Oxygen was measured as a function of time in the presence or absence of nitrate and in the presence of methanol as electron donor and carbon source. The incubation consisted of *Methylomonas* 16a cells suspended in HEPES buffer pH 7.

15     Methanol was injected at 3 min into both incubations to achieve a final concentration of about 100 mM. After the methanol injection it can be seen that oxygen uptake accelerated as would be expected (Figure 4) in the cultures without nitrate. However the rate of oxygen uptake in the presence of nitrate never approaches that of cells without nitrate. The data

20     thus supports the finding that nitrate can spare oxygen consumption with methanol as carbon source.

*Methylomonas* 16a cells were again suspended in HEPES buffer pH 7 and incubated in a water jacketed chamber equipped with an Orion oxygen probe. The incubation was carried out at 30°C. Methanol was

25     injected into the incubation at 1 min. However in one incubation sodium nitrite (25 mM) was injected into the incubation after 23 min. The results are shown in Figure 5. As seen in Figure 5, there is a decrease in the rate of oxygen uptake after the addition of nitrite. This data again clearly

30     supports the assertion that nitrite and indirectly nitrate can be used as an alternative electron sink and resulting in less oxygen consumption by the culture.

A cell suspension of *Methylobacter* 16a in defined medium under 25% methane in air was simultaneously monitored for oxygen and N<sub>2</sub>O in the dead-space. 100 mM Nitrite was the only added source of nitrogen. The results are shown in Figure 6. Figure 6 illustrates that the appearance of N<sub>2</sub>O in the dead-space coincides with oxygen depletion. The numbers plotted are the rates of appearance or disappearance of N<sub>2</sub>O and oxygen respectively. As oxygen disappearance rates decline to lower values (due to lower headspace O<sub>2</sub> concentrations) N<sub>2</sub>O production increases to become a significant fraction of the total electron flow through the organism (only under oxygen limitation).

#### EXAMPLE 9

#### NITRATE OR NITRITE REDUCTION BY OTHER STRAINS OF METHANOTROPHS AND METHYLOMONAS 16A.

All methanotrophic strains available from the American Type Culture collection were tested for their ability to produce N<sub>2</sub>O from nitrite or nitrate. All strains were grown on the defined medium and harvested after an optical density at 660 nm of 1.0 was achieved. The cell suspensions were collected by centrifugation and resuspended in 5 mL of defined medium with either nitrate or nitrite as sole nitrogen source. The data in Table 7 below shows the accumulation of N<sub>2</sub>O (in uM concentration) in the headspace of a 10 mL assay vial incubated 30°C. The results shows that *Methylobacter* 16a has a unique ability to convert nitrate to N<sub>2</sub>O among the strains tested. Furthermore the data show that two other *Methylobacter* strains have a similar ability to convert nitrite to N<sub>2</sub>O.

Table 7

STRAIN	NO <sub>3</sub> /NO <sub>2</sub> uM	NO <sub>2</sub> /N <sub>2</sub> O uM
<i>Methylobacter</i> 16a	28.3	30
<i>Methylobacter albus</i>	1.2	22
<i>Methylobacter clara</i>	2.5	1.5
<i>Methylobacter agile</i>	0.6	17
<i>Methylobacter whitterbury</i>	0.3	0.04
<i>Methylococcus capsulatis</i>	0.3	1.9
<i>Methylobacter lutes</i>	0.1	6.5
<i>Methylosinus sporium</i>	0.2	0.07

EXAMPLE 10  
PRODUCTION OF GLYCOGEN.

*Methylomonas* 16a was shown to accumulate large amounts of glycogen when grown on either methane or methanol. *Methylomonas* cells were analyzed for glycogen using a starch assay kit (Sigma Chemical Co. St Louis MO). This assay is starch or glycogen specific and conclusively shows the presence of glycogen in *Methylomonas* 16a. Cells were grown according to the conditions outlined in the General Methods, Cells were harvested during growth on 100 mM methanol or 25% headspace methane at 30°C on defined medium. Culture samples were taken at two points in the growth curve: mid-logarithmic growth (O.D. 660 0.3) and stationary phase (O.D. 660 1.0). These samples were immediately analyzed with the starch assay kit according to the manufacturers instructions. The results shown below in Table 8 indicate surprising amounts of the storage polymer during growth on methanol and lower but significant amounts of glycogen during growth on methane.

Table 8

<u>Growth Phase (OD660)</u>	<u>Methane (%glycogen wt/wt)</u>	<u>Methanol (% glycogen wt/wt)</u>
<u>Mid-log (0.3)</u>	<u>6%</u>	<u>25%</u>
<u>Stationary phase (1.0)</u>	<u>7%</u>	<u>40%</u>

Additionally, the presence of granules within the cells grown on methanol were observed by scanning electron microscopy and the granules were determined to contain starch with polysaccharide specific stains.

EXAMPLE 11  
PRODUCTION OF PROTEIN FROM CELL MASS

*Methylomonas* 16a and *Methylococcus capsulatus* (reference strain for protein production) were grown on defined medium until no further increases in OD 660 could be observed. Methane or methanol consumption was monitored by gas chromatography (HP-Plot Molecular sieve column; Hewlett Packard 5890 series II gas chromatograph) over the growth curve such that the total amount of methane or methanol consumed could be calculated. The running conditions for GC were; oven

temperature: 40°C, initial temperature: 40°C, initial time: 3 min, rate: 0 deg/min, final temperature 40°C, final time 0, injection A temperature: 100°C, Det. A temperature: 125°C, and equilibration time: 0.

The cells were collected by centrifugation and dried overnight in a 105°C drying oven. The data in Table 9 below shows the gram dry weight of cells produced per gram of methane or methanol consumed.

Table 9

Organism	g dry wt./g CH <sub>4</sub>	g dry wt./g CH <sub>4</sub> OH
<i>Methylomonas</i> 16a	0.90 - 1.3 (2-2.5 hr)	0.30 - 0.45 (2.5 - 3.0 hr)
<i>Methylococcus capsulatus</i>	0.67 - 1.2 (3 - 4 hr)	0.25 - 0.45 (4 -5 hr)

As can be seen by the data in Table 9 the present strain has a higher rate of protein production than the commercial methanotroph of choice for this process, when grown on either methane or methanol.

#### EXAMPLE 12

##### PRODUCTION OF EXTRACELLULAR POLYSACCHARIDE

*Methylomonas* 16a cells were grown on 25% methane in 200 mL batch culture on defined medium at 30°C. Initial oxygen concentration was varied by injecting pure oxygen into nitrogen flushed bottles. Cells were allowed to grow until stationary phase or to an optical density of approximately 1.0. At that time the cultures were centrifuged at 6000 x g for 30 min to sediment both the cells and the extracellular polysaccharide. The sediments from these centrifugations comprised two layers. At the bottom were the cells, overlaid with a clear viscous material which was the extracellular polysaccharide (EPS). The EPS layer was washed off and pelleted again for further separation from the cells. The cell pellet was also dried and weighed. The EPS was resuspended in 50% ethanol and pelleted again in the centrifuge. Finally the material was dried and weighed. EPS was found to comprise as much as 50% of the total dry weight of the culture at near-ambient oxygen concentrations. This was determined by centrifugation of the culture at 10,000 x g for 30 min. The resulting pellet is comprised of a lower red phase (packed cells) and an upper translucent phase which is the extracellular polysaccharide. The EPS was selectively removed with a spatula and dried at 105°C overnight. The cell pellet was removed and dried at 105°C overnight. The

supernatant from the centrifugation was mixed with cold isopropanol (1:1 vol:vol). The precipitated EPS from this step was collected by centrifugation (10,000 x g for 30 min) and the pellet dried at 105°C overnight and weighed. Chemical analysis of the EPS revealed that it was  
5 primarily polyglucose (~70%). EPS samples were methylated by the method of Ciucanu, I., F. Kerek. 1984. *Carbohydrate Research* 131:209-217. The methylated samples were hydrolyzed in 2 M TFA at 121°C for 2 hours and the hydrolyzed carbohydrate was reduced with sodium borodeuteride at room temperature. The product was acetylated  
10 by GC-MS using Sp2330 Supelco column. Internal standard myo-inositol was added to each sample prior to the reduction step.

CLAIMS

What is claimed is:

1. A high growth methanotrophic bacterial strain which:
  - (a) grows on a C1 carbon substrate selected from the group consisting of methane and methanol; and
  - (b) comprises a functional Embden-Meyerhof carbon pathway, said pathway comprising a gene encoding a pyrophosphate dependent phosphofructokinase enzyme, the gene selected from the group consisting of:
    - (a) an isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO:6;
    - (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS;
    - (c) an isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 437 amino acids that has at least 63% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:6; and
    - (d) an isolated nucleic acid molecule that is complementary to (a), (b) or (c).
2. A high growth methanotrophic bacterial strain according to Claim 1 wherein the strain optionally contains a functional Entner-Doudoroff carbon pathway.
3. A bacterial strain according to Claim 1 having at least one gene encoding a fructose biphosphate aldolase enzyme.
4. A bacterial strain according to Claim 3 wherein at least one gene encodes a fructose biphosphate aldolase enzyme having the amino acid sequence selected from the group consisting of SEQ ID NO:16 and SEQ ID NO:18.
5. A bacterial strain according to Claim 2 having at least one gene encoding a keto-deoxy phosphogluconate aldolase.
6. A bacterial strain according to Claim 5 wherein at least one gene encodes a keto-deoxy phosphogluconate aldolase enzyme is selected from the group consisting of:

- (a) an isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO:20;
- (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS;
- (c) an isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 212 amino acids that has at least 59% identity based on the Smith-Waterman method of alignment when compared to a polypeptide having the sequence as set forth in SEQ ID NO:20; and
- (d) an isolated nucleic acid molecule that is complementary to (a), (b) or (c).
7. A bacterial strain according to any of Claims 1 or 2 having a gene encoding a polypeptide involved in carbon flux wherein the polypeptide is selected from the group consisting of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18 and 20.
8. A bacterial strain according to any of Claims 1 or 2 optionally comprising a denitrifying enzymatic pathway.
9. The bacterial strain of Claim 8 wherein the enzymes of the denitrifying pathway are polypeptides having the amino acid sequences selected from the group consisting of SEQ ID NO:40, 42, 44, 46, 48, 50, 52, 54, 56, 58 and 60.
10. The bacterial strain of any of Claims 1 or 2 having genes encoding exopolysaccharide synthesizing enzymes, the enzymes selected from the group consisting of SEQ ID NO:22, 24, 26, 28, 30, 32, 34, 36, and 38.
11. The bacterial strain of any of Claims 1 or 2 having genes encoding isoprenoid synthesizing enzymes, the enzymes selected from the group consisting of SEQ ID NO:62, 64, 66, 68, 70, 72, 74, 86, and 78.
12. The bacterial strain of Claim 1 wherein the strain is a *Methylobacter* sp.
13. The bacterial strain of Claim 12 having a 16S RNA profile as set forth in SEQ ID NO:81.
14. The bacterial strain of Claim 1 wherein, when the C1 carbon substrate is methanol, the strain produces glycogen comprising at least about 50 % dry weight of biomass.

- 15 The bacterial strain of either Claim 1 or Claim 14 wherein the methanol concentration in the medium is about 2.5% (vol/vol).
16. The bacterial strain of any of Claims 1 or 2 having a yield of greater than 1.0 grams of cell mass per gram of methane consumed.
- 5 17. The bacterial strain of any of Claims 1 or 2 having a yield of greater than 0.5 grams of cell mass per gram of methane consumed.
18. The bacterial strain of any of Claims 1 or 2 having a carbon conversion efficiency of greater than 40 g/mol methane/g/ mol biomass.
19. The bacterial strain of any of Claims 1 or 2 having a carbon  
10 conversion efficiency of greater than 65 g/mol methane/g/ mol biomass.
20. The bacterial strain of any of Claims 1 or 2 having a carbon conversion efficiency of greater than 70 g/mol methane/g/ mol biomass.
21. A high growth methanotrophic bacterial strain which grows on a C1 carbon substrate selected from the group consisting of methanol and  
15 methane, comprising the 16s RNA sequence as set forth in SEQ ID NO:81 and having at least one gene encoding a pyrophosphate dependent Phosphofructokinase enzyme.
22. A high growth methanotrophic bacterial strain according to Claim 20 optionally having at least one gene encoding a keto-deoxy  
20 phosphogluconate aldolase.
23. A high growth methanotrophic bacterial strain having the ATCC designation PTA 2402.
24. A method for the production of single cell protein comprising:
- 25 a) contacting the bacterial strains of any of the Claims 1, 2, 3, 5, or 18 with C1 carbon substrate, selected from the group consisting of methane and methanol, in a suitable medium for a time sufficient to permit the expression and accumulation of single cell protein; and
- b) optionally recovering the single cell protein.
- 30 25. The method of Claim 23 wherein the C1 carbon substrate is contacted with the bacterial strain under anaerobic conditions.
26. The method of Claim 23 wherein the C1 carbon substrate is contacted with the bacterial strain under aerobic conditions.
27. A method for the biotransformation of a nitrogen containing  
35 compound selected from the group consisting of ammonia, nitrate, nitrite, and dinitrogen, comprising contacting the bacterial strain of any of the Claims 8 or 9 with a C1 carbon substrate selected from the group consisting of methane or methanol, in the presence of the nitrogen



containing compound, in a suitable medium for a time sufficient to permit the biotransformation of the nitrogen containing compound.

28. A method for the production of a feed product comprising protein, carbohydrates and pigment comprising the steps of:

- 5           a) contacting the bacterial strain of any of Claims 1, 2, 3, 5 or 18 with a C1 carbon substrate in a suitable medium for a time sufficient to permit the expression and accumulation of the feed product; and
- b) optionally recovering the feed product.

10           29. A method according to Claim 28 wherein the relative compositions of protein, carbohydrate and pigment are altered through the up-regulation or down-regulation of any one of the genes encoding the proteins selected from the group consisting of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51,  
15       53, 55, 57, 59, 61, 63, 65, 67, and 69.

30. A method of identifying the high growth methanotrophic bacterial strain of Claim 1 comprising:

- 20           (a) growing a sample suspected of containing a high growth methanotrophic bacterial strain on a suitable growth medium in the presence of methane as a sole carbon source;
- (b) identifying colonies that grow on the conditions of step (a);
- (c) analyzing the colonies identified in step (b) for the presence of pyrophosphate dependent  
25       phosphofructokinase activity.

31. A method according to Claim 30 wherein the colonies of step (b) are additionally analyzed for the presence of a gene selected from the group consisting of:

- 30           (a) an isolated nucleic acid molecule encoding the amino acid sequence as set forth in SEQ ID NO:6;
- (b) an isolated nucleic acid molecule that hybridizes with (a) under the following hybridization conditions: 0.1X SSC, 0.1% SDS, 65°C and washed with 2X SSC, 0.1% SDS followed by 0.1X SSC, 0.1% SDS;
- 35           (c) an isolated nucleic acid molecule comprising a first nucleotide sequence encoding a polypeptide of at least 437 amino acids that has at least 63% identity based on the Smith-Waterman method of alignment when

- compared to a polypeptide having the sequence as set forth in SEQ ID NO:6; and
- (d) an isolated nucleic acid molecule that is complementary to (a), (b) or (c).

Figure 1

## 16a vs. MCapsulatus Growth Curve

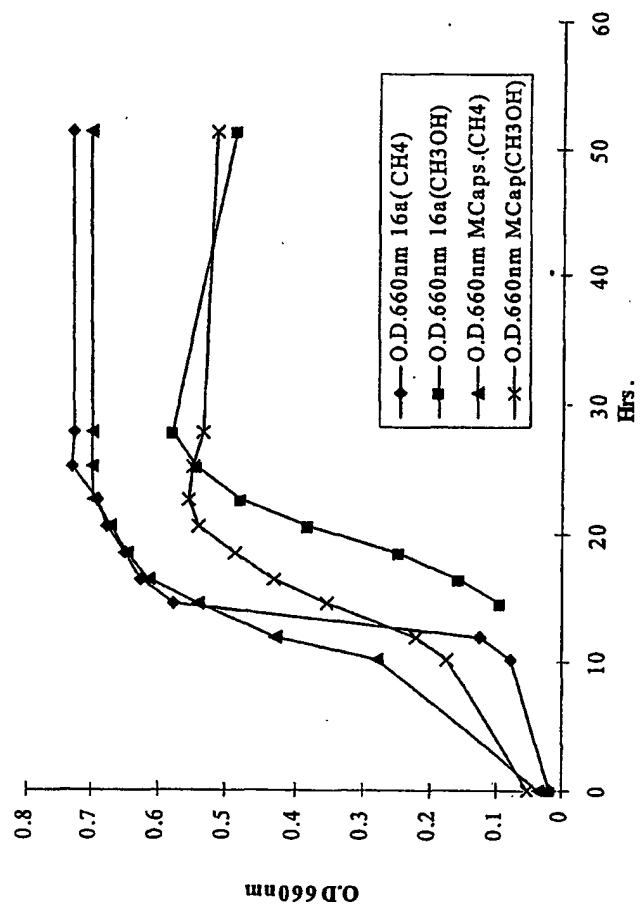


Figure 2

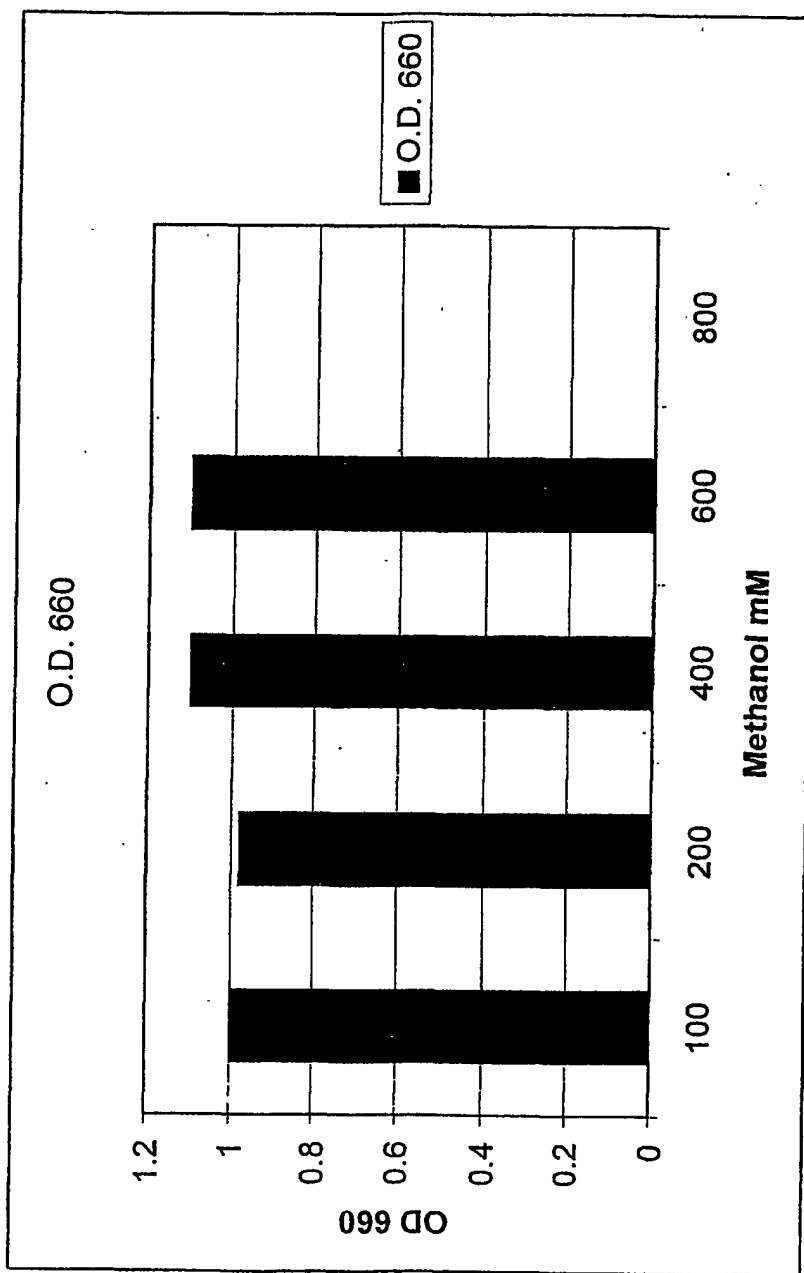


Figure 3

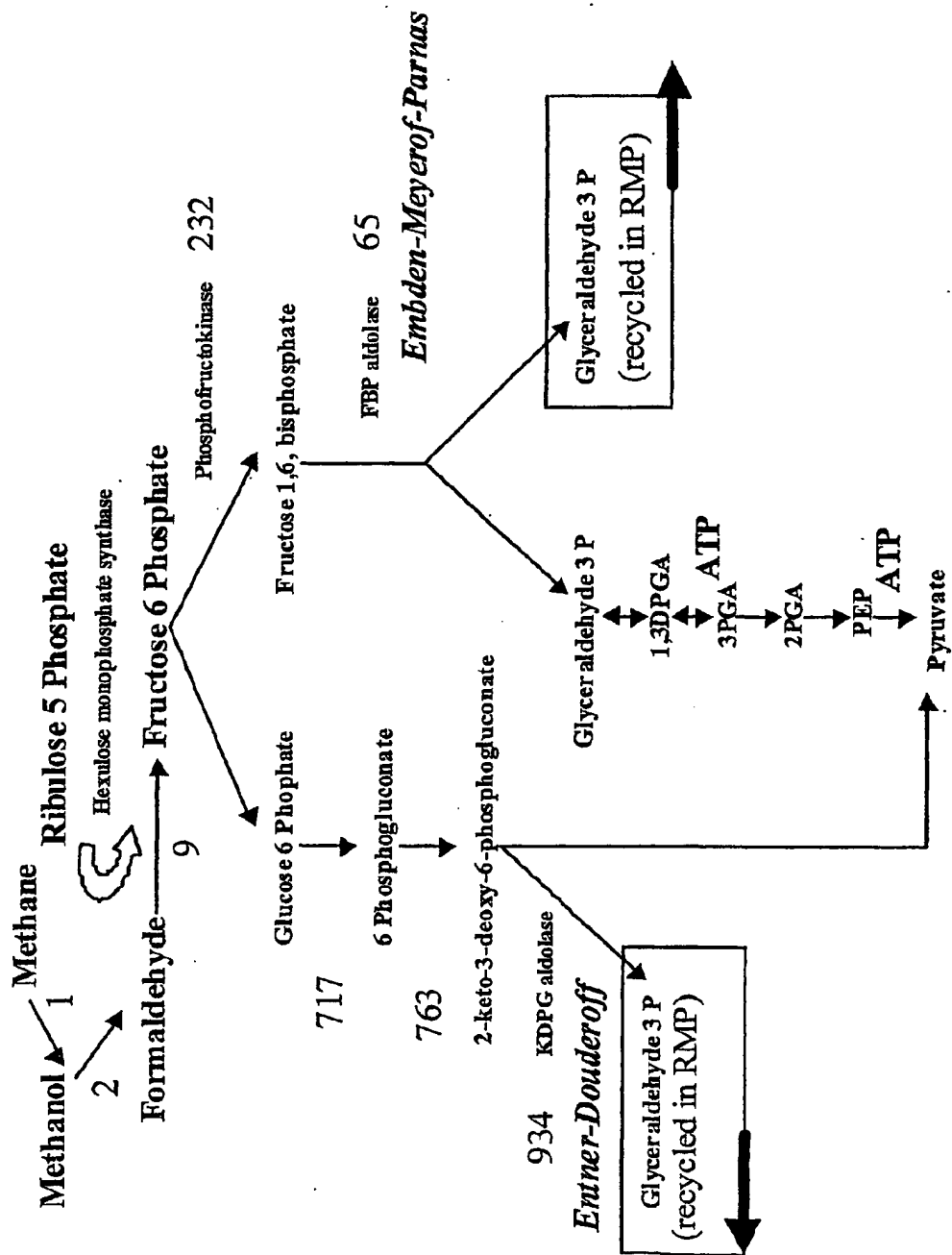


Figure 4

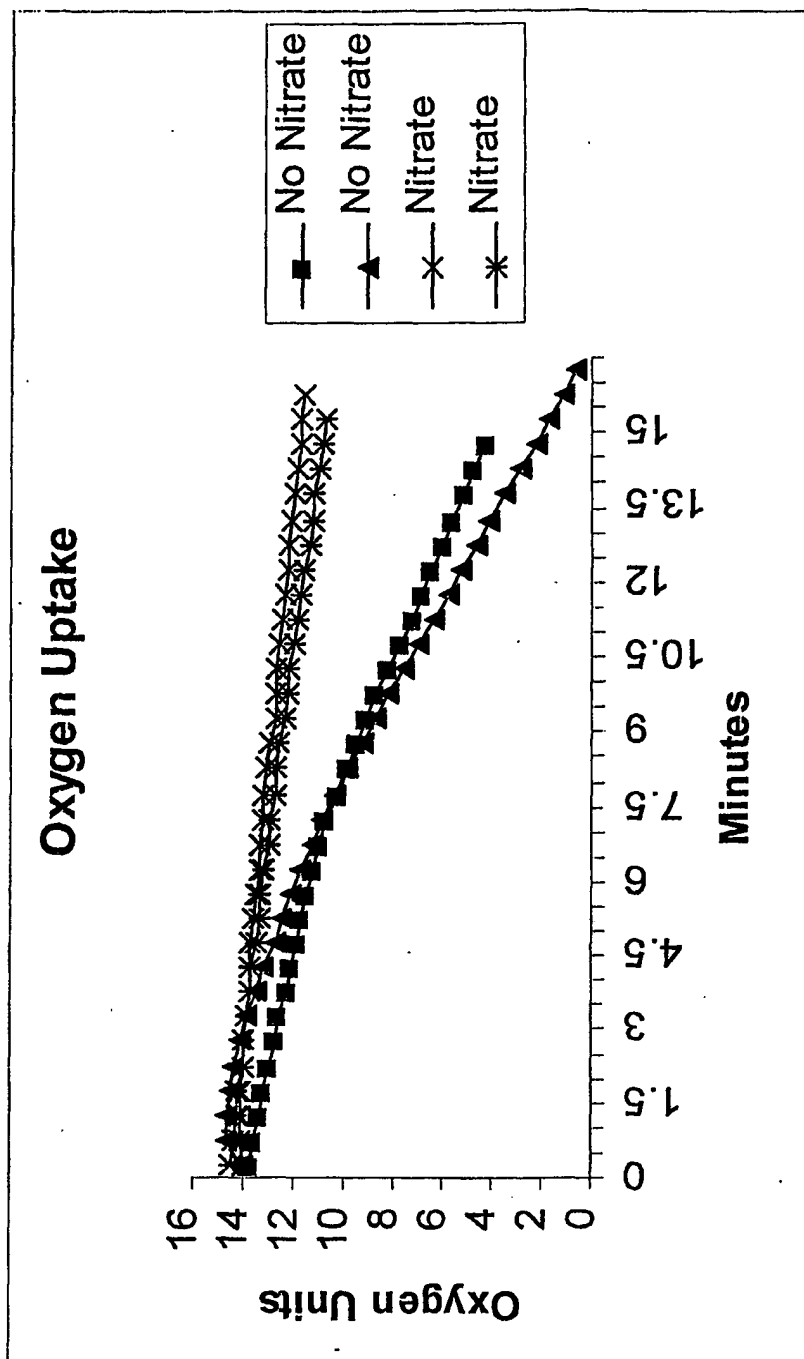


Figure 5

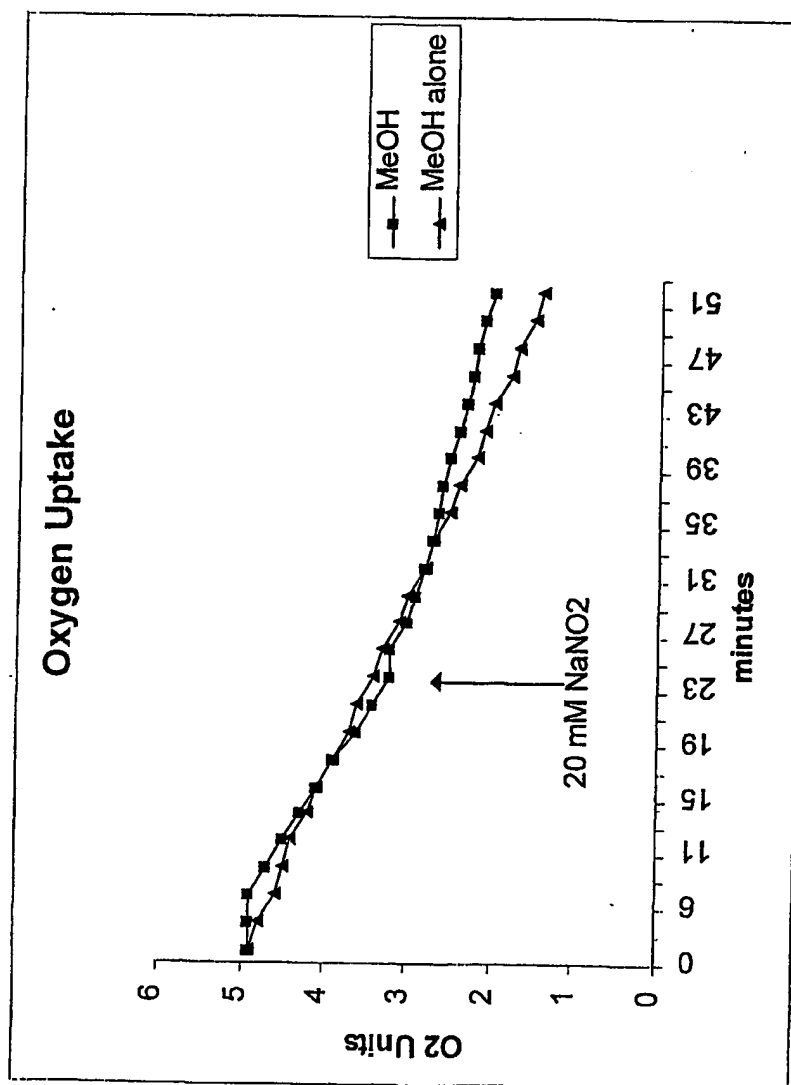
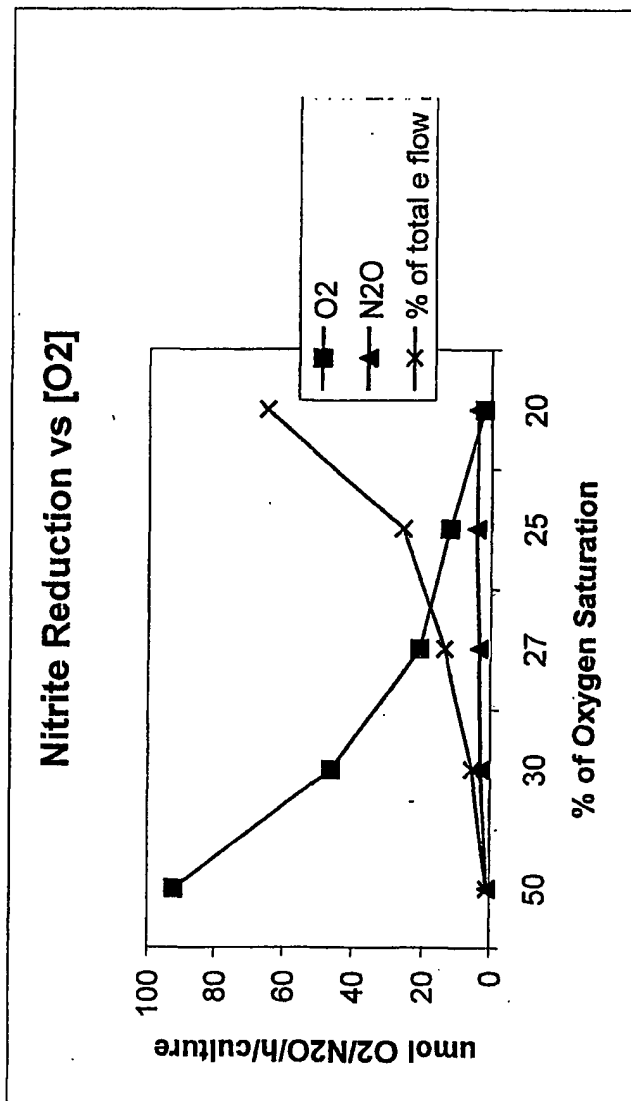


Figure 6





## SEQUENCE LISTING

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&lt;120&gt; DENITRIFYING METHANOTROPHIC BACTERIAL STRAIN

&lt;130&gt; CL1596

&lt;140&gt;

&lt;141&gt;

&lt;150&gt; 60/229,858

&lt;151&gt; September 1, 2000

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&lt;170&gt; Microsoft Office 97

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&lt;211&gt; 1434

&lt;212&gt; DNA

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&lt;211&gt; 545

&lt;212&gt; PRT

&lt;213&gt; METHYLOMONAS SP.

&lt;400&gt; 2

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Asn Met Gln Ile Lys Thr Tyr Lys Thr Thr Pro Tyr Asp Asp Gln Lys
  1                   5                   10                   15

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Pro Gly Thr Ser Gly Leu Arg Lys Lys Val Lys Val Phe Gln Gln Ser  
                   20                  25                  30  
 Gly Tyr Leu Glu Asn Phe Val Gln Ser Ile Phe Asn Ser Leu Glu Asp  
                   35                  40                  45  
 Phe Gln Gly Lys Ile Leu Val Leu Gly Gly Asp Gly Arg Tyr Phe Asn  
                   50                  55                  60  
 Arg Gln Ala Ile Gln Ile Ile Ile Lys Met ala Ala Ala Asn Gly Phe  
                   65                  70                  75                  80  
 Gly Glu Leu Ile Ile Gly Gln Gly Gly Leu Leu Ser Thr Pro Ala Ala  
                   85                  90                  95  
 Ser Asn Val Ile Arg Lys Tyr Arg Ala Phe Gly Gly Ile Ile Leu Ser  
                   100                  105                  110  
 Ala Ser His Asn Pro Gly Gly Pro Asp Glu Asp Phe Gly Ile Lys Tyr  
                   115                  120                  125  
 Asn Val Gly Asn Gly Gly Pro Ala Pro Glu Lys Phe Thr Asp Ala Leu  
                   130                  135                  140  
 Phe Glu Asn Ser Lys Thr Ile Thr Ser Tyr Gln Met ala Glu Ile Asp  
                   145                  150                  155                  160  
 Asp Ile Asp Leu Asp Ser Val Gly Asp Val Gln Ile Asp Gly Ile Thr  
                   165                  170                  175  
 Ile Arg Ile Ile Asp Pro Val Ala Asp Tyr Ala Glu Leu Met ala Arg  
                   180                  185                  190  
 Ile Phe Asp Phe Asp Leu Ile Lys Gln Ser Ile Ala Ala Gly Leu Ile  
                   195                  200                  205  
 Thr Leu Arg Phe Asp Ala Met His Ala Ile Thr Gly Pro Tyr Ala Lys  
                   210                  215                  220  
 His Ile Leu Glu Asp Val Leu Gly Ala Ala Pro Gly Ser Val Phe Asn  
                   225                  230                  235                  240  
 Ala Val Pro Leu Glu Asp Phe Gly Gly Gly His Pro Asp Pro Asn Met  
                   245                  250                  255  
 Ala His Ala His Glu Leu Thr Glu Ile Met Phe Gly Pro Asn Pro Pro  
                   260                  265                  270  
 Val Phe Gly Ala Ala Ser Asp Gly Asp Gly Asp Arg Asn Met Ile Met  
                   275                  280                  285  
 Gly Ala Asn Ile Phe Val Thr Pro Ser Asp Ser Leu Ala Ile Met ala  
                   290                  295                  300  
 Ala Asn Ala Gln Leu Ile Pro Ala Tyr Ala Lys Gly Ile Ser Gly Val  
                   305                  310                  315                  320  
 Ala Arg Ser Met Pro Thr Ser Gln Ala Val Asp Arg Val Ala Asp Lys  
                   325                  330                  335

Leu Ser Leu Pro Cys Tyr Glu Thr Pro Thr Gly Trp Lys Phe Phe Gly  
 340 345 350  
 Asn Leu Leu Asp Ala Asp Lys Ile Thr Leu Cys Gly Glu Glu Ser Phe  
 355 360 365  
 Gly Ser Gly Ser Asn His Val Arg Glu Lys Asp Gly Leu Trp Ala Val  
 370 375 380  
 Leu Phe Trp Leu Asn Leu Leu Ala Arg Lys Arg Gln Pro Ala Glu Asp  
 385 390 395 400  
 Ile Val Arg Glu His Trp Gln Lys Tyr Gly Arg Asp Ile Tyr Cys Arg  
 405 410 415  
 His Asp Tyr Glu Ala Val Asp Ala Asp Ile Ala Asn Gly Ile Val Glu  
 420 425 430  
 Gln Leu Arg Asn Gln Leu Pro Ser Leu Pro Gly Lys Thr Trp Gly Asp  
 435 440 445  
 Tyr Ser Val Lys Phe Ala Asp Glu Phe Ser Tyr Thr Asp Pro Val Asp  
 450 455 460  
 Gly Ser Val Ser Ser Asn Gln Gly Ile Arg Val Gly Phe Ala Asn Gly  
 465 470 475 480  
 Ser Arg Ile Val Phe Arg Leu Ser Gly Thr Gly Thr Val Gly Ala Thr  
 485 490 495  
 Leu Arg Ile Tyr Leu Glu Arg Tyr Glu Arg Asp Val Arg Asn His Asp  
 500 505 510  
 Gln Asp Pro Gln Val Ala Leu Ala Glu Leu Ile Glu Ile Ala Glu Gln  
 515 520 525  
 Leu Cys Gln Val Lys Gln Arg Thr Gly Arg Thr Glu Pro Ser Val Ile  
 530 535 540  
 Thr  
 545

&lt;210&gt; 3

&lt;211&gt; 1387

&lt;212&gt; DNA

&lt;213&gt; METHYLOMONAS SP.

&lt;400&gt; 3

ccgaaagcag gcaaaatcac gggttcatttt tttttgtcat ccgtcaaaga caatccttat 60  
 aatgaggtaa tcgttctcct cgctacatct ggcactaaag cttccgaaga ctctttatcc 120  
 gggtcacaca aaaataatat gtccaaatta atcaactctg ccgaatggaa cgccgtcaaa 180  
 caacatcatc aagaaattgc tggtaaatTT tgcattgaaag aggccttttgc caaagatccc 240  
 cagcggtttcg ataaattctc cgtcaccttt aacgacatat tattagacta ttccaaaaaac 300  
 ctgatcgacg agcgcacccat gcccttgctg atcgcattgg caaagcgggc agacttgccg 360  
 gagaaaacgg aagcgatgtt ttccggctcc atcatcaaca ccaccgaaaa acgcgcgggt 420  
 ttgcataccg cgctgcgaaa ccgtagcaat acgcccgttt tcttcgcgcg ccaggatgtc 480  
 atgccggaaa tcaacaaggt tctggcaaaa atgcggggtt tcgtggaaca ggtgcgttcg 540  
 ggccaatgga cgggctatag cggcaaggcc attaccgata tcgtcaacat cggcattggc 600  
 ggctcggatc tcggcccga aatggctcgac accgccttga cgcgtacgg caaaaacggc 660

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ttaaagcgc atttcgtatc caatgtcgat caaaccgaca tcgtcgaaac cctgaaaccg 720
ctcaatccgg aaaccacgct gttcctgatt tcatcgaaaa cgtttaccac gcaggaaacc 780
atgaccaatg cgcgctcggc acgtaactgg ttcatgaatg ccgcgcaaga tcccgcccat 840
atcaagaaac atttcatcgc catttccacc aacgaagaaa tggtaagga attcggcatc 900
gaccgcgga acatgttcga gttctgggac tgggtcggcg ggcgttattc gctctggtcg 960
gtcatcggca tgtcgatagc tttatatatc ggcattggaca atttcgaaga actgctgatg 1020
gggtgcgcaat tggccgacga acatttcgcg catgcgccct acgaggaaaa cattccggtc 1080
atcatgggct tgcctcgcat ctggtacaac aacttcttcg aagcggaaac ctatgccatt 1140
ttgccgtatg cgcaatcctt gaaatatatt gccgattatt tccagcaagg cgacatggaa 1200
agcaacggca aaagcgcgac gatcaccggt gaaaaagtgc attacaacac gggcccccac 1260
atctggggac agcccgccac caatggtcag cagccttct ttcaattgat tcaccaaggc 1320
accaactgg ttcccgccga tttctggcg gccgcgcaaa gtcagtatga tttaccggat 1380
caccacg

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&lt;210&gt; 4

&lt;211&gt; 592

&lt;212&gt; PRT

&lt;213&gt; METHYLOMONAS SP.

&lt;400&gt; 4

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Pro Lys Ala Gly Lys Ile Thr Val His Phe Phe Leu Ser Ser Val Lys
  1              5              10              15

Asp Asn Pro Tyr Asn Glu Val Ile Val Leu Leu Ala Thr Ser Gly Thr
      20              25              30

Lys Ala Ser Glu Asp Ser Leu Ser Gly Ser His Lys Asn Asn Met Ser
      35              40              45

Lys Leu Ile Asn Ser Ala Glu Trp Asn Ala Val Lys Gln His His Gln
      50              55              60

Glu Ile Ala Gly Lys Phe Cys Met Lys Glu Ala Phe Ala Lys Asp Pro
      65              70              75              80

Gln Arg Phe Asp Lys Phe Ser Val Thr Phe Asn Asp Ile Leu Leu Asp
      85              90              95

Tyr Ser Lys Asn Leu Ile Asp Glu Arg Thr Met Pro Leu Leu Ile Ala
      100              105              110

Leu Ala Lys Arg Ala Asp Leu Arg Glu Lys Thr Glu Ala Met Phe Ser
      115              120              125

Gly Ser Ile Ile Asn Thr Thr Glu Lys Arg Ala Val Leu His Thr Ala
      130              135              140

Leu Arg Asn Arg Ser Asn Thr Pro Val Phe Phe Arg Gly Gln Asp Val
      145              150              155              160

Met Pro Glu Ile Asn Lys Val Leu Ala Lys Met Arg Val Phe Val Glu
      165              170              175

Gln Val Arg Ser Gly Gln Trp Thr Gly Tyr Ser Gly Lys Ala Ile Thr
      180              185              190

Asp Ile Val Asn Ile Gly Ile Gly Gly Ser Asp Leu Gly Pro Lys Met
      195              200              205

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Val Asp Thr Ala Leu Thr Pro Tyr Gly Lys Asn Gly Leu Lys Ala His  
 210 215 220  
 Phe Val Ser Asn Val Asp Gln Thr Asp Ile Val Glu Thr Leu Lys Pro  
 225 230 235 240  
 Leu Asn Pro Glu Thr Thr Leu Phe Leu Ile Ser Ser Lys Thr Phe Thr  
 245 250 255  
 Thr Gln Glu Thr Met Thr Asn Ala Arg Ser Ala Arg Asn Trp Phe Met  
 260 265 270  
 Asn Ala Ala Gln Asp Pro Ala His Ile Lys Lys His Phe Ile Ala Ile  
 275 280 285  
 Ser Thr Asn Glu Glu Met Val Lys Glu Phe Gly Ile Asp Pro Ala Asn  
 290 295 300  
 Met Phe Glu Phe Trp Asp Trp Val Gly Gly Arg Tyr Ser Leu Trp Ser  
 305 310 315 320  
 Val Ile Gly Met Ser Ile Ala Leu Tyr Ile Gly Met Asp Asn Phe Glu  
 325 330 335  
 Glu Leu Leu Met Gly Ala His Leu Ala Asp Glu His Phe Arg His Ala  
 340 345 350  
 Pro Tyr Glu Glu Asn Ile Pro Val Ile Met Gly Leu Leu Gly Ile Trp  
 355 360 365  
 Tyr Asn Asn Phe Phe Glu Ala Glu Thr Tyr Ala Ile Leu Pro Tyr Ala  
 370 375 380  
 Gln Ser Leu Lys Tyr Phe Ala Asp Tyr Phe Gln Gln Gly Asp Met Glu  
 385 390 395 400  
 Ser Asn Gly Lys Ser Ala Thr Ile Thr Gly Glu Lys Val Asp Tyr Asn  
 405 410 415  
 Thr Gly Pro Ile Ile Trp Gly Gln Pro Gly Thr Asn Gly Gln His Ala  
 420 425 430  
 Phe Phe Gln Leu Ile His Gln Gly Thr Lys Leu Val Pro Gly Asp Phe  
 435 440 445  
 Leu Ala Ala Ala Gln Ser Gln Tyr Asp Leu Pro Asp His His Asp Ile  
 450 455 460  
 Leu Ile Ser Asn Phe Leu Ala Gln Ala Glu Ala Leu Met Arg Gly Lys  
 465 470 475 480  
 Thr Glu Glu Glu Val Arg Gln Asp Leu Ser His Glu Pro Asn Leu Asp  
 485 490 495  
 Asp Ala Leu Ile Ala Ser Lys Ile Phe Glu Gly Asn Lys Pro Ser Asn  
 500 505 510  
 Ser Phe Leu Phe Lys Lys Leu Thr Pro Arg Thr Leu Gly Thr Leu Ile  
 515 520 525

Ala Phe Tyr Glu His Lys Ile Phe Val Gln Gly Val Ile Trp Asn Ile  
530 535 540

Asn Ser Phe Asp Gln Met Gly Val Glu Leu Gly Lys Val Leu Ala Lys  
545 550 555 560

Ala Ile Leu Pro Glu Leu Lys Asn Asp Asp Ile Ile Ala Ser His Asp  
565 570 575

Ser Ser Thr Asn Gly Leu Ile Asn Thr Tyr Lys Arg Leu Arg Lys Ala  
580 585 590

<210> 5

<211> 1311

<212> DNA

<213> METHYLOMONAS SP.

<400> 5

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gatgtggtca catggcccta tcacttaacg gctgatattc gattttgtca ttggtttttt 60
cttaacttta acttctacac gctcatgaac aaacctaaaa aagttgcaat actgacagca 120
ggcggcttgg cgccttggtt gaattccgca atcggtagtt tgatcgaacg ttataccgaa 180
atcgatccta gcatagaaat catttgctat cgcggcggtt ataaaggcct gttgctgggc 240
gattcttata cagtaacggc cgaagtgcgt aaaaaggcgg gtgttctgca acgttttggc 300
ggttctgtga tcggcaacag ccgogtcaaa ttgaccaatg tcaaagactg cgtgaaacgc 360
ggtttggtca aagaggggtga agatccgcaa aaagtgcggg ctgatcaatt ggttaaggat 420
ggtgtcgata ttctgcacac catcggcggc gatgatacca atacggcagc agcggatttg 480
gcagcattcc tggccagaaa taattacgga ctgaccgtca ttggtttacc taaaaccgtc 540
gataacgacg tatttccgat caagcaatca ctagggtgctt ggactgccgc cgagcaaggc 600
gcgcggttatt tcatgaacgt ggtggccgaa aacaacgcca acccacgcat gctgatcgta 660
cacgaagtga tgggcccgtaa ctgcggtggt ctgaccgtg caaccgcgca ggaatatcgc 720
aaattactgg accgtgccga gtggttgccg gaattgggtt tgactcgtga atcttatgaa 780
gtgcacgcgg tattcggtcc ggaaatggcg atcgacctgg aagccgaagc caagcgctg 840
cgcgaaagtga tggacaaagt cgattgcgtc aacatcttcg ttccgaagg tgccggcgtc 900
gaagctatcg tcgcggaaat gcaggccaaa ggccaggaag tgccgcgcga tcggttcggc 960
cacatcaaac tggatgcggt caaccctggt aaatgggttcg gcgagcaatt cgcgcagatg 1020
ataggcgcgg aaaaaaccct ggtacaaaaa tcgggatact tcgcccgtgc ttctgcttcc 1080
aacggttgacg acatgcggtt gatcaaatcg tgcgcgact tggcggtcga gtgcgcgttc 1140
cgccgcgagt ctggcgtgat cggtcacgac gaagacaacg gcaacgtgtt gcgtgcgatac 1200
gagtttccgc gcatcaaggg cggcaaaccg ttcaatatcg acaccgactg gttcaatagc 1260
atgttgagcg aaatcggcca gcctaaaggc ggtaaagtcg aagtcagcca c 1311

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<210> 6

<211> 437

<212> PRT

<213> METHYLOMONAS SP.

<400> 6

Asp Val Val Thr Trp Pro Tyr His Leu Thr Ala Asp Ile Arg Phe Cys  
1 5 10 15

His Trp Phe Phe Leu Asn Phe Asn Phe Tyr Thr Leu Met Asn Lys Pro  
20 25 30

Lys Lys Val Ala Ile Leu Thr Ala Gly Gly Leu Ala Pro Cys Leu Asn  
35 40 45

Ser Ala Ile Gly Ser Leu Ile Glu Arg Tyr Thr Glu Ile Asp Pro Ser  
50 55 60

Ile Glu Ile Ile Cys Tyr Arg Gly Gly Tyr Lys Gly Leu Leu Leu Gly  
 65 70 75 80  
 Asp Ser Tyr Pro Val Thr Ala Glu Val Arg Lys Lys Ala Gly Val Leu  
 85 90 95  
 Gln Arg Phe Gly Gly Ser Val Ile Gly Asn Ser Arg Val Lys Leu Thr  
 100 105 110  
 Asn Val Lys Asp Cys Val Lys Arg Gly Leu Val Lys Glu Gly Glu Asp  
 115 120 125  
 Pro Gln Lys Val Ala Ala Asp Gln Leu Val Lys Asp Gly Val Asp Ile  
 130 135 140  
 Leu His Thr Ile Gly Gly Asp Asp Thr Asn Thr Ala Ala Ala Asp Leu  
 145 150 155 160  
 Ala Ala Phe Leu Ala Arg Asn Asn Tyr Gly Leu Thr Val Ile Gly Leu  
 165 170 175  
 Pro Lys Thr Val Asp Asn Asp Val Phe Pro Ile Lys Gln Ser Leu Gly  
 180 185 190  
 Ala Trp Thr Ala Ala Glu Gln Gly Ala Arg Tyr Phe Met Asn Val Val  
 195 200 205  
 Ala Glu Asn Asn Ala Asn Pro Arg Met Leu Ile Val His Glu Val Met  
 210 215 220  
 Gly Arg Asn Cys Gly Trp Leu Thr Ala Ala Thr Ala Gln Glu Tyr Arg  
 225 230 235 240  
 Lys Leu Leu Asp Arg Ala Glu Trp Leu Pro Glu Leu Gly Leu Thr Arg  
 245 250 255  
 Glu Ser Tyr Glu Val His Ala Val Phe Val Pro Glu Met ala Ile Asp  
 260 265 270  
 Leu Glu Ala Glu Ala Lys Arg Leu Arg Glu Val Met Asp Lys Val Asp  
 275 280 285  
 Cys Val Asn Ile Phe Val Ser Glu Gly Ala Gly Val Glu Ala Ile Val  
 290 295 300  
 Ala Glu Met Gln Ala Lys Gly Gln Glu Val Pro Arg Asp Ala Phe Gly  
 305 310 315 320  
 His Ile Lys Leu Asp Ala Val Asn Pro Gly Lys Trp Phe Gly Glu Gln  
 325 330 335  
 Phe Ala Gln Met Ile Gly Ala Glu Lys Thr Leu Val Gln Lys Ser Gly  
 340 345 350  
 Tyr Phe Ala Arg Ala Ser Ala Ser Asn Val Asp Asp Met Arg Leu Ile  
 355 360 365  
 Lys Ser Cys Ala Asp Leu Ala Val Glu Cys Ala Phe Arg Arg Glu Ser  
 370 375 380

Gly Val Ile Gly His Asp Glu Asp Asn Gly Asn Val Leu Arg Ala Ile  
385 390 395 400

Glu Phe Pro Arg Ile Lys Gly Gly Lys Pro Phe Asn Ile Asp Thr Asp  
405 410 415

Trp Phe Asn Ser Met Leu Ser Glu Ile Gly Gln Pro Lys Gly Gly Lys  
420 425 430

Val Glu Val Ser His  
435

<210> 7

<211> 1360

<212> DNA

<213> METHYLOMONAS SP.

<400> 7

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agtgtcccg actcgcatca cccggagaca tccttaatgc atcccgtact cgaaaaagtc 60
acagaacaag tcatcgccc cagccgggaa accdgtgccg cttatctgaa gcgcatagag 120
gccgcatcg ccgaaggccc gcaacgcaat aaactgcctt gcgccaatct ggcccacggt 180
ttcgcggtct gttcgcccat cgaaaaagaa gaattgtctc atggcccaa gcccaatgtc 240
ggcatcatct cggcctacaa cgacatgctg tccgcgcacg aaccctacaa ggattatcct 300
gccctgatca aacaggccgt gcgcgaagcc ggcggcgttg ctcaattcgc cggcggcgtg 360
cccgcgatgt gcgacggcgt caccagggc atgcccggca tggaattgtc gctattcagc 420
cgcgacgtca tcgcatgtc caccgcgac gccttggtc ataacatgtt cgacgcggcg 480
ctgtatctgg gcgtctgcga caagatcgta cccggtttgt tgatcggtgc attgagcttc 540
ggccatttgc cggccgtttt cttgccagcc ggcccatga ccagcgccct gtccaacaag 600
gaaaaatccc gtgcccgca aaaatacgcc gaaggtaaga tcggtgaaaa agaattgctg 660
gaatcggaag ccaagtctta ccacagccca ggcacctgca ctttctatgg caccgccaac 720
agcaaccaga tgatggtcga gatcatgggc ctgcacctgc ccggtagttc cttcatcaat 780
ccttacaccc cactgcgcga cgaactgacc aaggccgccg ccaggcaggt gttgaaattc 840
accgcgctgg gcaacgactt caggccaatc gcgcatgtga tcgacgaaaa agccatcatc 900
aatgccatca tcggcttgct ggcgaccggc ggctcgacca accataccat ccatattgatc 960
gcgattgccc gcgcccggcg catcatcatc aactgggacg atttcgacgc cctatccaaa 1020
gtcattccgt tgcgaccaa gatctatccg aacggcccgg ccgacgtcaa ccaattccag 1080
gcggccggcg gcatgagctt attgatacac gaactgctgg atcacggctt gttgcacggc 1140
gacatcctga ccataggcga ccagcgcggc atggcccaat acagtcaagt accgacgctg 1200
caagacggcc aattacaatg gcagcccgtt cctaccgcat cgcgcgatcc cgaaatcatc 1260
gccagcgtgg caaaccttt cgcgcgggtt ggtggcctgc atgtgatgca tggcaatctg 1320
ggcgcggcg tatccaagat ttccgcgctc tccgaagatc 1360

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<210> 8

<211> 618

<212> PRT

<213> METHYLOMONAS SP.

<400> 8

Ser Val Pro His Ser His His Pro Glu Thr Ser Leu Met His Pro Val  
1 5 10 15

Leu Glu Lys Val Thr Glu Gln Val Ile Ala Arg Ser Arg Glu Thr Arg  
20 25 30

Ala Ala Tyr Leu Lys Arg Ile Glu Ala Ala Ile Ala Glu Gly Pro Gln  
35 40 45



Arg Asn Lys Leu Pro Cys Ala Asn Leu Ala His Gly Phe Ala Val Cys  
 50 55 60  
 Ser Ala Ile Glu Lys Glu Glu Leu Ser His Gly Pro Lys Pro Asn Val  
 65 70 75 80  
 Gly Ile Ile Ser Ala Tyr Asn Asp Met Leu Ser Ala His Glu Pro Tyr  
 85 90 95  
 Lys Asp Tyr Pro Ala Leu Ile Lys Gln Ala Val Arg Glu Ala Gly Gly  
 100 105 110  
 Val Ala Gln Phe Ala Gly Gly Val Pro Ala Met Cys Asp Gly Val Thr  
 115 120 125  
 Gln Gly Met Pro Gly Met Glu Leu Ser Leu Phe Ser Arg Asp Val Ile  
 130 135 140  
 Ala Met Ser Thr Ala Ile Gly Leu Ala His Asn Met Phe Asp Ala Ala  
 145 150 155 160  
 Leu Tyr Leu Gly Val Cys Asp Lys Ile Val Pro Gly Leu Leu Ile Gly  
 165 170 175  
 Ala Leu Ser Phe Gly His Leu Pro Ala Val Phe Leu Pro Ala Gly Pro  
 180 185 190  
 Met Thr Ser Gly Leu Ser Asn Lys Glu Lys Ser Arg Ala Arg Gln Lys  
 195 200 205  
 Tyr Ala Glu Gly Lys Ile Gly Glu Lys Glu Leu Leu Glu Ser Glu Ala  
 210 215 220  
 Lys Ser Tyr His Ser Pro Gly Thr Cys Thr Phe Tyr Gly Thr Ala Asn  
 225 230 235 240  
 Ser Asn Gln Met Met Val Glu Ile Met Gly Leu His Leu Pro Gly Ser  
 245 250 255  
 Ser Phe Ile Asn Pro Tyr Thr Pro Leu Arg Asp Glu Leu Thr Lys Ala  
 260 265 270  
 Ala Ala Arg Gln Val Leu Lys Phe Thr Ala Leu Gly Asn Asp Phe Arg  
 275 280 285  
 Pro Ile Ala His Val Ile Asp Glu Lys Ala Ile Ile Asn Ala Ile Ile  
 290 295 300  
 Gly Leu Leu Ala Thr Gly Gly Ser Thr Asn His Thr Ile His Leu Ile  
 305 310 315 320  
 Ala Ile Ala Arg Ala Ala Gly Ile Ile Ile Asn Trp Asp Asp Phe Asp  
 325 330 335  
 Ala Leu Ser Lys Val Ile Pro Leu Leu Thr Lys Ile Tyr Pro Asn Gly  
 340 345 350  
 Pro Ala Asp Val Asn Gln Phe Gln Ala Ala Gly Gly Met Ser Leu Leu  
 355 360 365

Ile His Glu Leu Leu Asp His Gly Leu Leu His Gly Asp Ile Leu Thr  
 370 375 380  
 Ile Gly Asp Gln Arg Gly Met ala Gln Tyr Ser Gln Val Pro Thr Leu  
 385 390 395 400  
 Gln Asp Gly Gln Leu Gln Trp Gln Pro Gly Pro Thr Ala Ser Arg Asp  
 405 410 415  
 Pro Glu Ile Ile Ala Ser Val Ala Lys Pro Phe Ala Ala Gly Gly Gly  
 420 425 430  
 Leu His Val Met His Gly Asn Leu Gly Arg Gly Val Ser Lys Ile Ser  
 435 440 445  
 Ala Val Ser Glu Asp His Gln Val Val Thr Ala Pro Ala Met Val Phe  
 450 455 460  
 Asp Asp Gln Leu Asp Val Val Ala Ala Phe Lys Arg Gly Glu Leu Glu  
 465 470 475 480  
 Lys Asp Val Ile Val Val Leu Arg Phe Gln Gly Pro Lys Ala Asn Gly  
 485 490 495  
 Met Pro Glu Leu His Lys Leu Thr Pro Val Leu Gly Val Leu Gln Asp  
 500 505 510  
 Arg Gly Phe Lys Val Gly Leu Leu Thr Asp Gly Arg Met Ser Gly Ala  
 515 520 525  
 Ser Gly Lys Val Pro Ser Ala Ile His Met Trp Pro Glu Cys Ile Asp  
 530 535 540  
 Gly Gly Pro Leu Ala Lys Val Arg Asp Gly Asp Ile Ile Val Met Asn  
 545 550 555 560  
 Thr Gln Thr Gly Glu Val Asn Val Gln Val Asp Pro Ala Glu Phe Lys  
 565 570 575  
 Ala Arg Val Ala Glu Pro Asn His Ala Thr Gly His His Phe Gly Met  
 580 585 590  
 Gly Arg Glu Leu Phe Gly Ala Met Arg Ala Gln Ala Ser Thr Ala Glu  
 595 600 605  
 Thr Gly Ala Thr Asn Leu Phe Phe Val Asp  
 610 615

&lt;210&gt; 9

&lt;211&gt; 1477

&lt;212&gt; DNA

&lt;213&gt; METHYLOMONAS SP.

&lt;400&gt; 9

atggcattgg gcttttttgct ccgtagcccc aaagacatga caaaaaacat .tacttacaaa 60  
 ccctgcgacc tgggtgattta cggcgcaactg ggcgatttat ccaaacgtaa actactgatt 120  
 tcattatacc gtttggaata acacaatctg ctcgagcccg atacgcgcac catcggcgta 180  
 gatcgtttgg aagaaaccag cgacagtttc gtcgaaattg cgcacaaaag cttgcaggcg 240  
 tttttgaaca acgtcatcga cgcagaaatc tggcaacgtt tttccaaacg cttgtcctat 300

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ttgaaaatcg atctgaccca acccgagcaa tacaacaac tgcatacggc cgctgatgcc 360
gaaaaacgag tcatggtgaa ttatttcgcg gtggcaccct ttttggtcaa aaacatttgc 420
caaggcttgc atgactgcgg cgtattgacg gccgaatcgc gcatgggtgat ggaaaaaccc 480
atcggccacg acctgaaatc gtcgaaagaa atcaacgacg tcgtcgccga cgtattccac 540
gaagaccagg tctaccgcat cgaccactac ctgggcaagg aaacgggtctt gaacttgctg 600
gccttgcggtt tcgccaattc gatattcacg accaactgga atcacaacac gatagaccat 660
atccagatta cggtcggtga ggacatcggc atcgagggcc gttgggaata tttcgacaag 720
accggccaat tgcgcgacat gctgcaaac catttgctgc aaatcctgac cttcgctgcg 780
atggagccgc ccgcggatct gtcggccgaa agcatacaca tggaaaaaat caaggctctg 840
aaagccttgc ggccaatcac cgtgcgcaat gtcgaggaaa aaaccgtgcg cgggtcaatac 900
accgccgggtt tcatcaaagg caagtcggtg ccgggttatc tggaagaaga aggtgccaac 960
accgaaagca cgaccgaaac tttcgtcgcg atccgcgtgg atatcgataa ctggcgctgg 1020
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gtggtcaatt tcaagcaatt gccgcacaac atcttcaagg acagttttca tgaactgccg 1140
gccaataaac tggtcattca tttgcaaccg aacgaagggg tggatgtcat gatgttgaac 1200
aagggtgcgg gcatagacg caacatcaag ttgcaacaga ccaaactgga tttgagcttt 1260
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gtcgattcga ttcaggatgc ctggcaacac aaccacacgc cacccaaacc ctatcccgcg 1440
ggtagctggg gtccagtggc atcggtcgca ttactgg 1477

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&lt;210&gt; 10

&lt;211&gt; 501

&lt;212&gt; PRT

&lt;213&gt; METHYLOMONAS SP.

&lt;400&gt; 10

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Met ala Leu Gly Phe Leu Leu Arg Ser Pro Lys Asp Met Thr Lys Asn
  1             5             10             15

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```

Ile Thr Tyr Lys Pro Cys Asp Leu Val Ile Tyr Gly Ala Leu Gly Asp
          20             25             30

```

```

Leu Ser Lys Arg Lys Leu Leu Ile Ser Leu Tyr Arg Leu Glu Lys His
          35             40             45

```

```

Asn Leu Leu Glu Pro Asp Thr Arg Ile Ile Gly Val Asp Arg Leu Glu
          50             55             60

```

```

Glu Thr Ser Asp Ser Phe Val Glu Ile Ala His Lys Ser Leu Gln Ala
          65             70             75             80

```

```

Phe Leu Asn Asn Val Ile Asp Ala Glu Ile Trp Gln Arg Phe Ser Lys
          85             90             95

```

```

Arg Leu Ser Tyr Leu Lys Ile Asp Leu Thr Gln Pro Glu Gln Tyr Lys
          100            105            110

```

```

Gln Leu His Thr Val Val Asp Ala Glu Lys Arg Val Met Val Asn Tyr
          115            120            125

```

```

Phe Ala Val Ala Pro Phe Leu Phe Lys Asn Ile Cys Gln Gly Leu His
          130            135            140

```

```

Asp Cys Gly Val Leu Thr Ala Glu Ser Arg Met Val Met Glu Lys Pro
          145            150            155            160

```

```

Ile Gly His Asp Leu Lys Ser Ser Lys Glu Ile Asn Asp Val Val Ala
          165            170            175

```

Asp Val Phe His Glu Asp Gln Val Tyr Arg Ile Asp His Tyr Leu Gly  
 180 185 190  
 Lys Glu Thr Val Leu Asn Leu Leu Ala Leu Arg Phe Ala Asn Ser Ile  
 195 200 205  
 Phe Thr Thr Asn Trp Asn His Asn Thr Ile Asp His Ile Gln Ile Thr  
 210 215 220  
 Val Gly Glu Asp Ile Gly Ile Glu Gly Arg Trp Glu Tyr Phe Asp Lys  
 225 230 235 240  
 Thr Gly Gln Leu Arg Asp Met Leu Gln Asn His Leu Leu Gln Ile Leu  
 245 250 255  
 Thr Phe Val Ala Met Glu Pro Pro Ala Asp Leu Ser Ala Glu Ser Ile  
 260 265 270  
 His Met Glu Lys Ile Lys Val Leu Lys Ala Leu Arg Pro Ile Thr Val  
 275 280 285  
 Arg Asn Val Glu Glu Lys Thr Val Arg Gly Gln Tyr Thr Ala Gly Phe  
 290 295 300  
 Ile Lys Gly Lys Ser Val Pro Gly Tyr Leu Glu Glu Glu Gly Ala Asn  
 305 310 315 320  
 Thr Glu Ser Thr Thr Glu Thr Phe Val Ala Ile Arg Val Asp Ile Asp  
 325 330 335  
 Asn Trp Arg Trp Ala Gly Val Pro Phe Tyr Met Arg Thr Gly Lys Arg  
 340 345 350  
 Thr Pro Asn Lys Arg Thr Glu Ile Val Val Asn Phe Lys Gln Leu Pro  
 355 360 365  
 His Asn Ile Phe Lys Asp Ser Phe His Glu Leu Pro Ala Asn Lys Leu  
 370 375 380  
 Val Ile His Leu Gln Pro Asn Glu Gly Val Asp Val Met Met Leu Asn  
 385 390 395 400  
 Lys Val Pro Gly Ile Asp Gly Asn Ile Lys Leu Gln Gln Thr Lys Leu  
 405 410 415  
 Asp Leu Ser Phe Ser Glu Thr Phe Lys Lys Asn Arg Ile Phe Gly Gly  
 420 425 430  
 Tyr Glu Lys Leu Ile Leu Glu Ala Leu Arg Gly Asn Pro Thr Leu Phe  
 435 440 445  
 Leu Ser Arg Glu Glu Ile Glu Gln Ala Trp Thr Trp Val Asp Ser Ile  
 450 455 460  
 Gln Asp Ala Trp Gln His Asn His Thr Pro Pro Lys Pro Tyr Pro Ala  
 465 470 475 480  
 Gly Ser Trp Gly Pro Val Ala Ser Val Ala Leu Leu Ala Arg Asp Gly  
 485 490 495

Arg Ala Trp Glu Glu  
500

<210> 11  
<211> 984  
<212> DNA  
<213> METHYLOMONAS SP.

<400> 11  
atggcaagaa acttacttga gcaactccgc gagatgaccg ttgttgttgc cgataccggt 60  
gacatccagg cgatcgaaac cttcaagccg cgcgatgcaa cgaccaaccc gtctttgatc 120  
accgccgcgg cgcaaatgcc gcaatatcaa ggcatcgttg acgacacctt gaaaggtgcg 180  
cgtgcgacgt tgggtgccag cgcttcggct gccgaggtgg cttcattggc gttcgatcgt 240  
ttggcggttt ctttcggttt gaaaatccctg gaaatcatcg aaggtcgcgt ttccaccgag 300  
gttgatgcgc gtttgtctta tgacaccgaa ggcactattg ccaaaggccg ggatctgatc 360  
aaacaatacg aagctgcagg tgtttccaaa gagcgcgtac tgatcaaaat tgccgcgacc 420  
tgggaaggca tccaggcggc tgccgttttg gaaaaagaag gtattcacac caacttgacc 480  
ctgttggttcg gtctgcacca ggcgattgct tgtgccgaaa acggcattac cctgatttct 540  
ccgtttgtcg gccgtattct ggactggtac aaaaaagaca ctggccgcga ctcttatcct 600  
tccaacgaag atcctggcgt attgtctgta actgaagttt ataactacta caaaaaattt 660  
ggttataaaa ctgaagtcac gggcgcgagc ttccgtaaca tcggcgaaat caccgaattg 720  
gcgggttgcg atctgttgac catcgcgcct tctctgctgg ccgaactgca atccgttgaa 780  
gggtgatttgc cacgcaaact ggaccctgca aaagcagccg gttcttcgat cgaaaaaatc 840  
agcgttgaca aagcgacttt cgagcgcgtg cacgaagaaa accgcatggc caaagaaaaa 900  
ctggccgaag gtatcgacgg ttttgcgaaa gcgttggaag ccttggaagaa attgttggcg 960  
gatcggttgg ctgctctgga agca 984

<210> 12  
<211> 328  
<212> PRT  
<213> METHYLOMONAS SP.

<400> 12  
Met ala Arg Asn Leu Leu Glu Gln Leu Arg Glu Met Thr Val Val Val  
1 5 10 15  
Ala Asp Thr Gly Asp Ile Gln Ala Ile Glu Thr Phe Lys Pro Arg Asp  
20 25 30  
Ala Thr Thr Asn Pro Ser Leu Ile Thr Ala Ala Ala Gln Met Pro Gln  
35 40 45  
Tyr Gln Gly Ile Val Asp Asp Thr Leu Lys Gly Ala Arg Ala Thr Leu  
50 55 60  
Gly Ala Ser Ala Ser Ala Ala Glu Val Ala Ser Leu Ala Phe Asp Arg  
65 70 75 80  
Leu Ala Val Ser Phe Gly Leu Lys Ile Leu Glu Ile Ile Glu Gly Arg  
85 90 95  
Val Ser Thr Glu Val Asp Ala Arg Leu Ser Tyr Asp Thr Glu Gly Thr  
100 105 110  
Ile Ala Lys Gly Arg Asp Leu Ile Lys Gln Tyr Glu Ala Ala Gly Val  
115 120 125

Ser Lys Glu Arg Val Leu Ile Lys Ile Ala Ala Thr Trp Glu Gly Ile  
 130 135 140

Gln Ala Ala Ala Val Leu Glu Lys Glu Gly Ile His Thr Asn Leu Thr  
 145 150 155 160

Leu Leu Phe Gly Leu His Gln Ala Ile Ala Cys Ala Glu Asn Gly Ile  
 165 170 175

Thr Leu Ile Ser Pro Phe Val Gly Arg Ile Leu Asp Trp Tyr Lys Lys  
 180 185 190

Asp Thr Gly Arg Asp Ser Tyr Pro Ser Asn Glu Asp Pro Gly Val Leu  
 195 200 205

Ser Val Thr Glu Val Tyr Asn Tyr Tyr Lys Lys Phe Gly Tyr Lys Thr  
 210 215 220

Glu Val Met Gly Ala Ser Phe Arg Asn Ile Gly Glu Ile Thr Glu Leu  
 225 230 235 240

Ala Gly Cys Asp Leu Leu Thr Ile Ala Pro Ser Leu Leu Ala Glu Leu  
 245 250 255

Gln Ser Val Glu Gly Asp Leu Pro Arg Lys Leu Asp Pro Ala Lys Ala  
 260 265 270

Ala Gly Ser Ser Ile Glu Lys Ile Ser Val Asp Lys Ala Thr Phe Glu  
 275 280 285

Arg Met His Glu Glu Asn Arg Met ala Lys Glu Lys Leu Ala Glu Gly  
 290 295 300

Ile Asp Gly Phe Ala Lys Ala Leu Glu Thr Leu Glu Lys Leu Leu Ala  
 305 310 315 320

Asp Arg Leu Ala Ala Leu Glu Ala  
 325

<210> 13  
 <211> 480  
 <212> DNA  
 <213> METHYLOMONAS SP.

<400> 13  
 atggccgcgg gcggcggtggg cttgacgcaa ttgctgccag aactggccga agctattggt 60  
 ccgacgagcc gatttcattgt gcaggtcatt ggtgacacgg tggaggacat cgttgccgaa 120  
 gccaaacggc tacacgattt gcccgtcgac atagtgggtga aaattccggc gcatggcgcc 180  
 ggactggcgg ccatcaagca gatcaagcgc cacgatattc cgggtgctggc gacagcgatt 240  
 tacaacgtgc agcaaggttg gctggcggct ttgaacggcg ccgattatct ggcgccttat 300  
 ctgaatcgcg tcgataacca gggttttgac ggtattggcg tggtcgccga tctgcagagc 360  
 ttgatcgacc ggtatcaaat gccaccaa ctctggttag cgagcttcaa aaacgtacaa 420  
 caggtgctgc aggtgttgaa actgggcgtg gcgtcggtga cgctgccttt ggacattgtg 480

<210> 14  
 <211> 160  
 <212> PRT  
 <213> METHYLOMONAS SP.

&lt;400&gt; 14

Met ala Ala Gly Gly Val Gly Leu Thr Gln Leu Leu Pro Glu Leu Ala  
 1 5 10 15

Glu Ala Ile Gly Pro Thr Ser Arg Phe His Val Gln Val Ile Gly Asp  
 20 25 30

Thr Val Glu Asp Ile Val Ala Glu Ala Lys Arg Leu His Asp Leu Pro  
 35 40 45

Val Asp Ile Val Val Lys Ile Pro Ala His Gly Ala Gly Leu Ala Ala  
 50 55 60

Ile Lys Gln Ile Lys Arg His Asp Ile Pro Val Leu Ala Thr Ala Ile  
 65 70 75 80

Tyr Asn Val Gln Gln Gly Trp Leu Ala Ala Leu Asn Gly Ala Asp Tyr  
 85 90 95

Leu Ala Pro Tyr Leu Asn Arg Val Asp Asn Gln Gly Phe Asp Gly Ile  
 100 105 110

Gly Val Val Ala Asp Leu Gln Ser Leu Ile Asp Arg Tyr Gln Met Pro  
 115 120 125

Thr Lys Leu Leu Val Ala Ser Phe Lys Asn Val Gln Gln Val Leu Gln  
 130 135 140

Val Leu Lys Leu Gly Val Ala Ser Val Thr Leu Pro Leu Asp Ile Val  
 145 150 155 160

&lt;210&gt; 15

&lt;211&gt; 1005

&lt;212&gt; DNA

&lt;213&gt; METHYLOMONAS SP.

&lt;400&gt; 15

atggcttttag tgtcattgcg acaacttttg gattatgcgg ccgagcatgg ctttgccgtg 60  
 ccggcggttca acgtcagcaa catggagcag gtacaggcca tcatgcaggc ggccgctgcc 120  
 tgcgatagtc cagtgatcat gcaagggttcg gcgggcgcca accgctatgc cggcgaagtg 180  
 tttctacggc atttgatatt ggcgccgtg gagcaatata cgcataattcc ggtcgtcatg 240  
 caccgcgacc atgcaccac gcccgacatc tgcgcgcaag ccatacaatc gggcttcagc 300  
 tcggtgatga tggacggttc gttgctggca gacatgaaaa ccccggttc ttttgcatatc 360  
 aacgtcgacg tccccgcac cgtggtcaag atggcgcatg cctgcggtgt atcgtgtgaa 420  
 ggcgaaatcg gctgcctggg agcgctggag gccaaagtccg cgcaagatca cagccgtttg 480  
 ctgaccgatc ccgacgaagc ggtcgaattc gtcgaacaga cccaggtcga tgccgtggcc 540  
 gtggccatcg gcaccagcca cggcgcttat aaattcagca agccgcccac cggcgaagtg 600  
 ctggtgatca gtcgattgaa agaactgcag caacgactgc caaataccca ttttgtgatg 660  
 catggctcca gttcggtgcc gcaggattgg ttgaaaatca tcaacgatta tggcggcgat 720  
 attccggaaa cctatggcgt gccggtcgaa gaaatcgtcg aaggcataaa atatggtgtg 780  
 cgcaaggtca acatcgatac cgacctgcgc atggogtcca ccggcgcgat gcgcaggttt 840  
 ctggcccaac cggaaaacgc ctccggagcta gacgcgcgca agacctatca agccgccagg 900  
 gacgcaatgc aggcattatg ccaggctcgc tacgaagcgt tcggttcggc gggacatgcc 960  
 ggcaaaatca aaccggtttc actggcggca atggccaaac gctat 1005

&lt;210&gt; 16

&lt;211&gt; 335

&lt;212&gt; PRT

&lt;213&gt; METHYLOMONAS SP.

&lt;400&gt; 16

Met ala Leu Val Ser Leu Arg Gln Leu Leu Asp Tyr Ala Ala Glu His  
 1 5 10 15

Gly Phe Ala Val Pro Ala Phe Asn Val Ser Asn Met Glu Gln Val Gln  
 20 25 30

Ala Ile Met Gln Ala Ala Ala Ala Cys Asp Ser Pro Val Ile Met Gln  
 35 40 45

Gly Ser Ala Gly Ala Asn Arg Tyr Ala Gly Glu Val Phe Leu Arg His  
 50 55 60

Leu Ile Leu Ala Ala Val Glu Gln Tyr Pro His Ile Pro Val Val Met  
 65 70 75 80

His Arg Asp His Ala Pro Thr Pro Asp Ile Cys Ala Gln Ala Ile Gln  
 85 90 95

Ser Gly Phe Ser Ser Val Met Met Asp Gly Ser Leu Leu Ala Asp Met  
 100 105 110

Lys Thr Pro Ala Ser Phe Ala Tyr Asn Val Asp Val Thr Arg Thr Val  
 115 120 125

Val Lys Met ala His Ala Cys Gly Val Ser Val Glu Gly Glu Ile Gly  
 130 135 140

Cys Leu Gly Ala Leu Glu Ala Lys Ser Ala Gln Asp His Ser Arg Leu  
 145 150 155 160

Leu Thr Asp Pro Asp Glu Ala Val Glu Phe Val Glu Gln Thr Gln Val  
 165 170 175

Asp Ala Val Ala Val Ala Ile Gly Thr Ser His Gly Ala Tyr Lys Phe  
 180 185 190

Ser Lys Pro Pro Thr Gly Glu Val Leu Val Ile Ser Arg Leu Lys Glu  
 195 200 205

Leu Gln Gln Arg Leu Pro Asn Thr His Phe Val Met His Gly Ser Ser  
 210 215 220

Ser Val Pro Gln Asp Trp Leu Lys Ile Ile Asn Asp Tyr Gly Gly Asp  
 225 230 235 240

Ile Pro Glu Thr Tyr Gly Val Pro Val Glu Glu Ile Val Glu Gly Ile  
 245 250 255

Lys Tyr Gly Val Arg Lys Val Asn Ile Asp Thr Asp Leu Arg Met ala  
 260 265 270

Ser Thr Gly Ala Met Arg Arg Phe Leu Ala Gln Pro Glu Asn Ala Ser  
 275 280 285



Glu Leu Asp Ala Arg Lys Thr Tyr Gln Ala Ala Arg Asp Ala Met Gln  
 290 295 300

Ala Leu Cys Gln Ala Arg Tyr Glu Ala Phe Gly Ser Ala Gly His Ala  
 305 310 315 320

Gly Lys Ile Lys Pro Val Ser Leu Ala Ala Met ala Lys Arg Tyr  
 325 330 335

<210> 17

<211> 1074

<212> DNA

<213> METHYLOMONAS SP.

<400> 17

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atgacaaaaa tcttagatgt tgtaaaaccc ggcgttgatca ccggtgaaga tgtgcaaaaa 60
attttcgcaa tctgcaaaga aaacaacttt gccttgccag ccgtcaacgt gatcagtacc 120
gataccatta atgcggtatt ggaagcggcc gccaaagcca aatcacctgt tgttatccag 180
ttttcaaata gcggcgcggc tttcgttgcc ggtaaaagggt tgaaattgga aggtcaaggc 240
tgttcgattc atggtgccat ttcaggtgct caccacgttc accgcttgcc ggaactctat 300
ggtgtacctg tcgttctgca taccgaccac gcggcgaaaa aattgctgcc atgggtagat 360
ggtatgctgg atgaagggtga aaaattcttt gcggccaccg gcaagccttt gtccagctcg 420
cacatgctgg acttgtccga agagagcctg gaagaaaaca tcgaaatctg cggtaaatac 480
ttggcgcgca tggcgaaaaat gggtagacc ttggaaatcg aactgggctg caccggcggt 540
gaagaagacg gcgtggacaa cagcggcatg gatcattccg cgttgtagac ccagccggaa 600
gacgtggctt acgctgatga gcacctgagc aaaatcagcc ctaacttcac gattgcggct 660
tctttcgcca acgtgcacgg cgtttactcg ccaaggaaacg tcaagctgac gccaaaaatt 720
ctggataact cgcaaaaata cgtatccgaa aaattcggct tgccagctaa atcattgacc 780
ttcgtattcc atggcggtc tggttcgtct ccggaagaaa tcaaggaatc catcagctat 840
ggcgtagtga aaatgaacat cgataccgat acccaatggg caacctggga aggcgtgatg 900
aacttctaca agaaaaacga aggctatctg caaggccaga tcggcaatcc ggaagggtgc 960
gacaagccga acaaaaaata ctatgacca cgcgtagtgc aacgtgccgg ccaagaaggc 1020
atggttgcac gtctgcaaca agcattccag gaattgaatg cagtaaacac gctg 1074

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<210> 18

<211> 358

<212> PRT

<213> METHYLOMONAS SP.

<400> 18

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Met Thr Lys Ile Leu Asp Val Val Lys Pro Gly Val Val Thr Gly Glu
  1 5 10 15
Asp Val Gln Lys Ile Phe Ala Ile Cys Lys Glu Asn Asn Phe Ala Leu
  20 25 30
Pro Ala Val Asn Val Ile Ser Thr Asp Thr Ile Asn Ala Val Leu Glu
  35 40 45
Ala Ala Ala Lys Ala Lys Ser Pro Val Val Ile Gln Phe Ser Asn Gly
  50 55 60
Gly Ala Ala Phe Val Ala Gly Lys Gly Leu Lys Leu Glu Gly Gln Gly
  65 70 75 80
Cys Ser Ile His Gly Ala Ile Ser Gly Ala His His Val His Arg Leu
  85 90 95

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Ala Glu Leu Tyr Gly Val Pro Val Val Leu His Thr Asp His Ala Ala  
 100 105 110  
 Lys Lys Leu Leu Pro Trp Val Asp Gly Met Leu Asp Glu Gly Glu Lys  
 115 120 125  
 Phe Phe Ala Ala Thr Gly Lys Pro Leu Phe Ser Ser His Met Leu Asp  
 130 135 140  
 Leu Ser Glu Glu Ser Leu Glu Glu Asn Ile Glu Ile Cys Gly Lys Tyr  
 145 150 155 160  
 Leu Ala Arg Met ala Lys Met Gly Met Thr Leu Glu Ile Glu Leu Gly  
 165 170 175  
 Cys Thr Gly Gly Glu Glu Asp Gly Val Asp Asn Ser Gly Met Asp His  
 180 185 190  
 Ser Ala Leu Tyr Thr Gln Pro Glu Asp Val Ala Tyr Ala Tyr Glu His  
 195 200 205  
 Leu Ser Lys Ile Ser Pro Asn Phe Thr Ile Ala Ala Ser Phe Gly Asn  
 210 215 220  
 Val His Gly Val Tyr Ser Pro Gly Asn Val Lys Leu Thr Pro Lys Ile  
 225 230 235 240  
 Leu Asp Asn Ser Gln Lys Tyr Val Ser Glu Lys Phe Gly Leu Pro Ala  
 245 250 255  
 Lys Ser Leu Thr Phe Val Phe His Gly Gly Ser Gly Ser Ser Pro Glu  
 260 265 270  
 Glu Ile Lys Glu Ser Ile Ser Tyr Gly Val Val Lys Met Asn Ile Asp  
 275 280 285  
 Thr Asp Thr Gln Trp Ala Thr Trp Glu Gly Val Met Asn Phe Tyr Lys  
 290 295 300  
 Lys Asn Glu Gly Tyr Leu Gln Gly Gln Ile Gly Asn Pro Glu Gly Ala  
 305 310 315 320  
 Asp Lys Pro Asn Lys Lys Tyr Tyr Asp Pro Arg Val Trp Gln Arg Ala  
 325 330 335  
 Gly Gln Glu Gly Met Val Ala Arg Leu Gln Gln Ala Phe Gln Glu Leu  
 340 345 350  
 Asn Ala Val Asn Thr Leu  
 355

&lt;210&gt; 19

&lt;211&gt; 636

&lt;212&gt; DNA

&lt;213&gt; METHYLOMONAS SP.

<400> 19  
 gaaaatacta tgtccgtcac catcaaagaa gtcattgacca cctcgcccgt tatgcccgtc 60  
 atgggtcatca atcatctgga acatgccgtc cctctgggtc gcgcgctagt cgacgggtggc 120  
 ttgaaagttt tggagatcac attgcgcacg ccggtggcac tggatgtat ccgacgtatc 180  
 aaagccgaag taccggagcg catcgtcggc gcgggcacca tcatcaaccc tcataccttg 240  
 tatcaagcga ttgacgccgg tgcggaattc atcgtcagcc ccggcatcac cgaataatcta 300  
 ctcaacgaag cgctagcatc cggcgtgcct atcctggccg gcgtcatcac acccagcgag 360  
 gtcattgcgtt tattggaaaa aggcattcaat gcgatgaaat tctttccggc tgaagccgcc 420  
 ggccgcatac cgatgctgaa atcccttggc ggccccttgc cgcaagtcac cttctgtccg 480  
 accggcggcg tcaatcccaa aaacgcgccc gaatatctgg cattgaaaaa tgtcgcctgc 540  
 gtcggcggct cctggatggc gccggccgat ctggtagatg ccgaagactg ggcggaaatc 600  
 acgcgccggg cgagcgaggc cgcggcattg aaaaaa 636

<210> 20  
 <211> 212  
 <212> PRT  
 <213> METHYLOMONAS SP.

<400> 20  
 Glu Asn Thr Met Ser Val Thr Ile Lys Glu Val Met Thr Thr Ser Pro  
 1 5 10 15  
 Val Met Pro Val Met Val Ile Asn His Leu Glu His Ala Val Pro Leu  
 20 25 30  
 Ala Arg Ala Leu Val Asp Gly Gly Leu Lys Val Leu Glu Ile Thr Leu  
 35 40 45  
 Arg Thr Pro Val Ala Leu Glu Cys Ile Arg Arg Ile Lys Ala Glu Val  
 50 55 60  
 Pro Asp Ala Ile Val Gly Ala Gly Thr Ile Ile Asn Pro His Thr Leu  
 65 70 75 80  
 Tyr Gln Ala Ile Asp Ala Gly Ala Glu Phe Ile Val Ser Pro Gly Ile  
 85 90 95  
 Thr Glu Asn Leu Leu Asn Glu Ala Leu Ala Ser Gly Val Pro Ile Leu  
 100 105 110  
 Pro Gly Val Ile Thr Pro Ser Glu Val Met Arg Leu Leu Glu Lys Gly  
 115 120 125  
 Ile Asn Ala Met Lys Phe Phe Pro Ala Glu Ala Ala Gly Gly Ile Pro  
 130 135 140  
 Met Leu Lys Ser Leu Gly Gly Pro Leu Pro Gln Val Thr Phe Cys Pro  
 145 150 155 160  
 Thr Gly Gly Val Asn Pro Lys Asn Ala Pro Glu Tyr Leu Ala Leu Lys  
 165 170 175  
 Asn Val Ala Cys Val Gly Gly Ser Trp Met ala Pro Ala Asp Leu Val  
 180 185 190  
 Asp Ala Glu Asp Trp Ala Glu Ile Thr Arg Arg Ala Ser Glu Ala Ala  
 195 200 205

Ala Leu Lys Lys  
210

<210> 21  
<211> 873  
<212> DNA  
<213> Methylomonas 16a

<400> 21  
atgaaagtta ccaaagccgt ttttcccgtt gccggactgg gcacccggtc attgcccgca 60  
accaaggccg ttgccaagga aatgttgccg gtgggtggaca agccgctgat tcagtatgcg 120  
gtggaagagg ccgtggccgc cggcatcgac acgatgattt tcgtgatcgg tagaaacaag 180  
gaatccattg ccaaccattt cgataaatcc tacgaactgg aaaaggaact ggaaaaaagc 240  
ggcaagaccg atttgtgaa aatgctgcgg gagattttgc ccgcgcattg gtcctgcgta 300  
ttcgtgcgtc aagcggaggc tctgggtttg gggcatgcgg tgcattgcgc caagccggtg 360  
gtcggcaacg agccgtttgc ggtgatcttg ccggatgact tgatcgagga cggcgagcgc 420  
ggttgcatga agcagatggt ggattttgtt gacaaagagc aaagcagcgt attgggggta 480  
gagcgggtcg atcccaagga aaccataaag tacggcatcg tcgaacatgc cgaaacctcg 540  
cccagagtcg gttggttgag ttccatcgtc gagaaacca aaccgaagt ggcgcctcc 600  
aatatcgccg tggtcgggcg ctacatcttg acgccggcca tttttcaaaa aatcgagaac 660  
acggggcgcg gcgccggcgg cgaaattcaa ttgaccgatg cgattgccgc gttgatgaaa 720  
gacgaacgcg ttttgtccta tgaattcgaa ggcaatcgct acgactgcgg ttccaagttt 780  
ggttttttgt tggccaatgt cgaatatggc ttgctgcaca aggaaatcaa agccgaattc 840  
gccaaactatc tgaacaacg cgtcagcaaa atc 873

<210> 22  
<211> 293  
<212> PRT  
<213> Methylomonas 16a

<400> 22  
Met Thr Met Lys Val Thr Lys Ala Val Phe Pro Val Ala Gly Leu Gly  
1 5 10 15  
Thr Arg Ser Leu Pro Ala Thr Lys Ala Val Ala Lys Glu Met Leu Pro  
20 25 30  
Val Val Asp Lys Pro Leu Ile Gln Tyr Ala Val Glu Glu Ala Val Ala  
35 40 45  
Ala Gly Ile Asp Thr Met Ile Phe Val Ile Gly Arg Asn Lys Glu Ser  
50 55 60  
Ile Ala Asn His Phe Asp Lys Ser Tyr Glu Leu Glu Lys Glu Leu Glu  
65 70 75 80  
Lys Ser Gly Lys Thr Asp Leu Leu Lys Met Leu Arg Glu Ile Leu Pro  
85 90 95  
Ala His Val Ser Cys Val Phe Val Arg Gln Ala Glu Ala Leu Gly Leu  
100 105 110  
Gly His Ala Val His Cys Ala Lys Pro Val Val Gly Asn Glu Pro Phe  
115 120 125  
Ala Val Ile Leu Pro Asp Asp Leu Ile Glu Asp Gly Glu Arg Gly Cys  
130 135 140

Met Lys Gln Met Val Asp Leu Phe Asp Lys Glu Gln Ser Ser Val Leu  
 145 150 155 160

Gly Val Glu Arg Val Asp Pro Lys Glu Thr His Lys Tyr Gly Ile Val  
 165 170 175

Glu His Ala Glu Thr Ser Pro Arg Val Gly Trp Leu Ser Ser Ile Val  
 180 185 190

Glu Lys Pro Lys Pro Glu Val Ala Pro Ser Asn Ile Ala Val Val Gly  
 195 200 205

Arg Tyr Ile Leu Thr Pro Ala Ile Phe Gln Lys Ile Glu Asn Thr Gly  
 210 215 220

Arg Gly Ala Gly Gly Glu Ile Gln Leu Thr Asp Ala Ile Ala Ala Leu  
 225 230 235 240

Met Lys Asp Glu Arg Val Leu Ser Tyr Glu Phe Glu Gly Asn Arg Tyr  
 245 250 255

Asp Cys Gly Ser Lys Phe Gly Phe Leu Leu Ala Asn Val Glu Tyr Gly  
 260 265 270

Leu Leu His Lys Glu Ile Lys Ala Glu Phe Ala Asn Tyr Leu Lys Gln  
 275 280 285

Arg Val Ser Lys Ile  
 290

&lt;210&gt; 23

&lt;211&gt; 1419

&lt;212&gt; DNA

&lt;213&gt; Methylobionas 16a

&lt;400&gt; 23

atgccactcg gtttgggaaa tatcttcaac gggctgttca agcaatacgg gcacacgggtg 60  
 atcctgttgt tgagggttat cgacgtggtc atgttattgg gcgcggcctg gctggcgcat 120  
 tatttttgggt tgcattgacag cgctcatcgat cagcattacc gtttcgtgat tgccctgggt 180  
 atcttgggtg cgatcatatt ttctgagatc ggccagggtg atcggccgtg gcgcaatgac 240  
 gcgatgcgcg gcgaaattcc ccgcatcatc agagcctggt tgctggcctt gctgacgggtg 300  
 gtgtccatcg tggccctggt cagattgcat ttttggtttg gttccagtta tcgctggatc 360  
 gcctcctggg gcggtttggg gctgttcttc gtactggcgg cccgcggtgt gctggcacag 420  
 gtgttgaaagt ggttgcggtc acggggctgg agccaggggc gcatcattct ggtgggtttg 480  
 aatcagatgg ccgtcgccgt cagtcggcaa ttgaatact cttcctgggc cggtttgacg 540  
 gtgattggtt atgtcgatga ccgggcccga gaccggctgg cgggtggcga ttattcgctg 600  
 ccacgcctgg gcaagttgag cgatctgcct cgtctggttt ccagacaagc cgtggatgaa 660  
 gtctgggtgg cgtttccctg cgcttcgctg gccgagcggg tacagcacga attgcgccat 720  
 ttgccggtca gcattcgctt ggtgatcgat tgctttgcct ttaaacaag caaatcctc 780  
 agtctgaaca cgggtggcgg tatcccagcg ctggacgtct cgggtgtcgc gctgcatggc 840  
 gtcaatcgct atatcaagga aatcgaggac cgcttgctgg ccttgctgtt gttgttgctg 900  
 atcagcccgt tgatgctggt cattgcgctt ggctgaaac tgagttctcc gggcccgggtg 960  
 ttttacaagc aggtcagagt gggctggaac aatcgcaaat tcacgatgct gaagtttctg 1020  
 tcgatgccgg tcgatgccga ggccaaaacc ggccgaggtc gggccaggcc cggcgaaaac 1080  
 cgtgaacccc ggtttggggc cttcctgcgc aaaaccagtc tggacgagtt gccgcagttg 1140  
 atcaatgtgc tcaagggcga catgtcgctg gtcggcccgc gccctgaacg gcccgatttc 1200  
 gtgcaggtgt tcaaggatca agtaccatc tacatgaaaa aacacatggt caaggcgggc 1260  
 attaccggtt gggcacaagt caacggctgg cgcggtgata ccgacctgaa tcgccgcatc 1320

gaacacgata tgtattacat ccagcattgg tcggtctggt tcgatctgga gattgccttt 1380  
cgcaccggtg tgaccggctt tatcaacaaa aatgcctat 1419

<210> 24

<211> 473

<212> PRT

<213> Methylomonas 16a

<400> 24

Met Pro Leu Gly Leu Gly Asn Ile Phe Asn Gly Leu Phe Lys Gln Tyr  
1 5 10 15

Gly His Thr Val Ile Leu Leu Leu Arg Val Ile Asp Val Val Met Leu  
20 25 30

Leu Gly Ala Ala Trp Leu Ala His Tyr Phe Trp Leu His Asp Ser Val  
35 40 45

Ile Asp Gln His Tyr Arg Phe Val Ile Ala Leu Gly Ile Leu Gly Ala  
50 55 60

Ile Ile Phe Phe Glu Ile Gly Gln Val Tyr Arg Pro Trp Arg Asn Asp  
65 70 75 80

Ala Met Arg Gly Glu Ile Pro Arg Ile Ile Arg Ala Trp Leu Leu Ala  
85 90 95

Leu Leu Thr Val Val Ser Ile Val Ala Leu Val Arg Leu His Phe Trp  
100 105 110

Phe Gly Ser Ser Tyr Arg Trp Ile Ala Ser Trp Gly Gly Leu Gly Leu  
115 120 125

Phe Phe Val Leu Ala Ala Arg Gly Val Leu Ala Gln Val Leu Lys Trp  
130 135 140

Leu Arg Ala Arg Gly Trp Ser Gln Gly Arg Ile Ile Leu Val Gly Leu  
145 150 155 160

Asn Gln Met ala Val Ala Val Ser Arg Gln Leu Asn His Ser Ser Trp  
165 170 175

Ala Gly Leu Gln Val Ile Gly Tyr Val Asp Asp Arg Ala Glu Asp Arg  
180 185 190

Leu Ala Val Ala Asp Tyr Ser Leu Pro Arg Leu Gly Lys Leu Ser Asp  
195 200 205

Leu Pro Arg Leu Val Ser Arg Gln Ala Val Asp Glu Val Trp Val Ala  
210 215 220

Phe Pro Gly Ala Ser Leu Ala Glu Arg Val Gln His Glu Leu Arg His  
225 230 235 240

Leu Pro Val Ser Ile Arg Leu Val Ile Asp Cys Phe Ala Phe Lys Gln  
245 250 255

Ser Lys Phe Leu Ser Leu Asn Thr Val Ala Gly Ile Pro Thr Leu Asp  
260 265 270

Val Ser Val Ser Pro Leu His Gly Val Asn Arg Tyr Ile Lys Glu Ile  
 275 280 285  
 Glu Asp Arg Leu Leu Ala Leu Leu Leu Leu Leu Ile Ser Pro Leu  
 290 295 300  
 Met Leu Val Ile Ala Leu Gly Val Lys Leu Ser Ser Pro Gly Pro Val  
 305 310 315 320  
 Phe Tyr Lys Gln Val Arg Val Gly Trp Asn Asn Arg Lys Phe Thr Met  
 325 330 335  
 Leu Lys Phe Arg Ser Met Pro Val Asp Ala Glu Ala Lys Thr Gly Ala  
 340 345 350  
 Val Trp Ala Arg Pro Gly Glu Asn Arg Ala Thr Arg Phe Gly Ala Phe  
 355 360 365  
 Leu Arg Lys Thr Ser Leu Asp Glu Leu Pro Gln Leu Ile Asn Val Leu  
 370 375 380  
 Lys Gly Asp Met Ser Leu Val Gly Pro Arg Pro Glu Arg Pro Asp Phe  
 385 390 395 400  
 Val Glu Val Phe Lys Asp Gln Val Pro Asn Tyr Met Lys Lys His Met  
 405 410 415  
 Val Lys Ala Gly Ile Thr Gly Trp Ala Gln Val Asn Gly Trp Arg Gly  
 420 425 430  
 Asp Thr Asp Leu Asn Arg Arg Ile Glu His Asp Leu Tyr Tyr Ile Gln  
 435 440 445  
 His Trp Ser Val Trp Phe Asp Leu Glu Ile Ala Phe Arg Thr Val Leu  
 450 455 460  
 Thr Gly Phe Ile Asn Lys Asn Ala Tyr  
 465 470

&lt;210&gt; 25

&lt;211&gt; 1098

&lt;212&gt; DNA

&lt;213&gt; Methylomonas 16a

&lt;400&gt; 25

```

atgttttagac taattcccat catgctgggt ttactgttgc caggctgttt cctggcaccg 60
ggtatggata tgcagaccga tggcgacttg acagaaatcg agctgccaac catgaagggc 120
gggcagttgg tcaaggagaa aaccgcgatt cagccgatca ccgccgattt gatcatcgag 180
cgtgaagtgc cacggcggca agccgtcaac aatctaccgc cgatggacga aaccgggacc 240
agttatcgca tcggtccgca ggacaggttg caaatcacgg tatgggagca tcccgaactg 300
aacgatcccg gcggcgagaa aatcctgccg gaactggccg gcaaggtcgt ggacgataac 360
ggcgatttgt attacccta tgtcggtacc cttcatgtcg gcggcaagac cgtcaccgaa 420
gtgcgcgagg aattgaccgg cgaactgtcc aaatacttca aaaaggtaa actcgacatt 480
cgtgtgtgtg cgttccaggc tcaccgcgtc gcggtggtcg gtgaagtcag aaatcccggc 540
atcgtcgca tgaccgaaac gccgttgacg gtggcagaag ccatcagcag gccggcgggc 600
gccacgcaag attccgattt gaacaacgtc gcgctggccc gcggcgggcg gttgtacaaa 660
ctggatgtgc aagccttgta tgaaaaaggc ctgaccacgc aaaacctgct gttgcgggat 720
ggcgatgtgc tgaacgtcgg cgatcagaaa gacagcaagg tttatgtgat gggcgaggtc 780

```

```

ggccggcagc aggccatcca gatcaacaag ggccggatga gtctggctca ggcgctggcc 840
gaagcctatg gcgtcgattt caacacctcg cgtcccggcg atatttacgt gctgcgcgcc 900
ggcgacatgc agccggagat tttccagctg gacgccgaat cgcccgcgcg gatgatcctg 960
gccgagcaat tcccgttgca gccgcacgac acgctattcg tcggtacggc cggggtcacg 1020
caatgggtcca ggggtgctgaa tcagattctg ccgggttcgt ttaccgccat catgtcgcaa 1080
gccgcgatga tgggggatg                                     1098

```

&lt;210&gt; 26

&lt;211&gt; 366

&lt;212&gt; PRT

&lt;213&gt; Methylobionas 16a

&lt;400&gt; 26

```

Met Phe Arg Leu Ile Pro Ile Met Leu Val Leu Leu Leu Pro Gly Cys
  1               5               10               15

Phe Leu Ala Pro Gly Met Asp Met Gln Thr Asp Gly Asp Leu Thr Glu
      20               25               30

Ile Glu Leu Pro Thr Met Lys Gly Gly Gln Leu Val Lys Glu Lys Thr
      35               40               45

Arg Ile Gln Pro Ile Thr Ala Asp Leu Ile Ile Glu Arg Glu Val Ala
      50               55               60

Arg Arg Gln Ala Val Asn Asn Leu Pro Pro Met Asp Glu Thr Arg Thr
      65               70               75               80

Ser Tyr Arg Ile Gly Pro Gln Asp Arg Leu Gln Ile Thr Val Trp Glu
      85               90               95

His Pro Glu Leu Asn Asp Pro Gly Gly Glu Lys Ile Leu Pro Glu Leu
      100               105               110

Ala Gly Lys Val Val Asp Asp Asn Gly Asp Leu Tyr Tyr Pro Tyr Val
      115               120               125

Gly Thr Leu His Val Gly Gly Lys Thr Val Thr Glu Val Arg Glu Glu
      130               135               140

Leu Thr Arg Glu Leu Ser Lys Tyr Phe Lys Lys Val Lys Leu Asp Ile
      145               150               155               160

Arg Val Leu Ser Phe Gln Ala His Arg Val Ala Val Val Gly Glu Val
      165               170               175

Arg Asn Pro Gly Ile Val Ala Met Thr Glu Thr Pro Leu Thr Val Ala
      180               185               190

Glu Ala Ile Ser Arg Ala Gly Gly Ala Thr Gln Asp Ser Asp Leu Asn
      195               200               205

Asn Val Ala Leu Ala Arg Gly Gly Arg Leu Tyr Lys Leu Asp Val Gln
      210               215               220

Ala Leu Tyr Glu Lys Gly Leu Thr Thr Gln Asn Leu Leu Leu Arg Asp
      225               230               235               240

```



Gly Asp Val Leu Asn Val Gly Asp Gln Lys Asp Ser Lys Val Tyr Val  
 245 250 255

Met Gly Glu Val Gly Arg Gln Gln Ala Ile Gln Ile Asn Lys Gly Arg  
 260 265 270

Met Ser Leu Ala Gln Ala Leu Ala Glu Ala Tyr Gly Val Asp Phe Asn  
 275 280 285

Thr Ser Arg Pro Gly Asp Ile Tyr Val Leu Arg Ala Gly Asp Met Gln  
 290 295 300

Pro Glu Ile Phe Gln Leu Asp Ala Glu Ser Pro Asp Ala Met Ile Leu  
 305 310 315 320

Ala Glu Gln Phe Pro Leu Gln Pro His Asp Thr Leu Phe Val Gly Thr  
 325 330 335

Ala Gly Val Thr Gln Trp Ser Arg Val Leu Asn Gln Ile Leu Pro Gly  
 340 345 350

Ser Phe Thr Ala Ile Met Ser Gln Ala Ala Met Met Gly Met  
 355 360 365

&lt;210&gt; 27

&lt;211&gt; 2337

&lt;212&gt; DNA

&lt;213&gt; Methylobionas 16a

&lt;400&gt; 27

```

atgccgcct tgaatccgt gatgatgcag gagcctggcg tcagcatccg cgattatgtc 60
gatctgttga tcgagggcaa gaagacaata ctgttgacgt tggccatcgt gctgagcgtg 120
acgatgattt atttggtttt ggccccgcgc acttacaagg ccgatgcctt gctgcgtatc 180
gacaaaaata aagccttggt ggcggccaat ttgcgtagcg agggcaatgg tacgccaacg 240
gaggcggaata accccagggc gcaacgggaa gtggaaattt tgcgctcgcg ttccgtgctg 300
ggcaagggtg tggaggattt gaatctagtc gtggaggcgt cgccacgata ctttcccatc 360
atcggcgaaa ccctggcccg caagcacgac aaacatgagg gcgtagccgg cgccctgggtg 420
ggattcagcc gttgggcctg gggcggggaa aaactgaaaa tcgagcggtt cgagggtgcc 480
gatcgttacc tggacaaggc ttttactttg gtggcgctgg aagcaggggc ttttcaatta 540
ttgagcccta agggcgaggt gctggccgaa ggtttgctcg gtgaaacgct gaccgcccag 600
atcggcgaag ccagtcccgt cgtcgtcaac gtcgctgatt tgcaggcgca ttacggcacc 660
gagttcagat tgcggcgcaa aacctcgctg gcggccatag aaacctgca aaaagccttt 720
tcggtcaagg aagtgtccaa ggataccaat attctgagtg tcgaactcaa ggggcgcgat 780
cccagagcaat tggccaaatc ggtcaacgac atcgccagta ttacgtcaa cgccacgggtg 840
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tccttcatca gcaccaattt ggcggctctg ttggccagca tacgcaagcg ggtgctgatc 1740

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gagataggcg tggatttgat tcccagggcg gagatgggtc tgaatccggc cgaattgttg 1920
gtgctgggcg atctggccga taccttgag caactgaaga gcttttacia ccatatcgctc 1980
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caaaccacgg cttggcaggc gcgctttcaa aacctgaatg actggatggg gcggcaggac 2280
gccgagtatt taccgctcgc cgacgacgcg gaagaacttc acgacagcat caggggcc 2337

```

&lt;210&gt; 28

&lt;211&gt; 779

&lt;212&gt; PRT

&lt;213&gt; Methylomonas 16a

&lt;400&gt; 28

```

Met Pro Pro Leu Asn Pro Val Met Met Gln Glu Pro Gly Val Ser Ile
  1              5              10              15

Arg Asp Tyr Val Asp Leu Leu Ile Glu Gly Lys Lys Thr Ile Leu Leu
      20              25              30

Thr Leu Ala Ile Val Leu Ser Val Thr Met Ile Tyr Leu Val Leu Ala
      35              40              45

Pro Arg Thr Tyr Lys Ala Asp Ala Leu Leu Arg Ile Asp Lys Asn Lys
      50              55              60

Ala Leu Leu Ala Ala Asn Leu Arg Ser Glu Gly Asn Gly Thr Pro Thr
      65              70              75              80

Glu Ala Glu Asn Pro Arg Ala Gln Arg Glu Val Glu Ile Leu Arg Ser
      85              90              95

Arg Ser Val Leu Gly Lys Val Val Glu Asp Leu Asn Leu Val Val Glu
      100              105              110

Ala Ser Pro Arg Tyr Phe Pro Ile Ile Gly Glu Thr Leu Ala Arg Lys
      115              120              125

His Asp Lys His Glu Gly Val Ala Gly Ala Trp Trp Gly Phe Ser Arg
      130              135              140

Trp Ala Trp Gly Gly Glu Lys Leu Lys Ile Glu Arg Phe Glu Val Pro
      145              150              155              160

Asp Arg Tyr Leu Asp Lys Ala Phe Thr Leu Val Ala Leu Glu Ala Gly
      165              170              175

Arg Phe Gln Leu Leu Ser Pro Lys Gly Glu Val Leu Ala Glu Gly Leu
      180              185              190

Leu Gly Glu Thr Leu Thr Ala Asp Ile Gly Glu Ala Ser Pro Val Val
      195              200              205

Val Asn Val Ala Asp Leu Gln Ala His Tyr Gly Thr Glu Phe Glu Leu
      210              215              220

```

Arg Arg Lys Thr Ser Leu Ala Ala Ile Glu Thr Leu Gln Lys Ala Phe  
 225 230 235 240  
 Ser Val Lys Glu Val Ser Lys Asp Thr Asn Ile Leu Ser Val Glu Leu  
 245 250 255  
 Lys Gly Arg Asp Pro Glu Gln Leu Ala Lys Ser Val Asn Asp Ile Ala  
 260 265 270  
 Ser Ile Tyr Val Asn Ala Thr Val Asn Trp Glu Ser Ala Glu Ala Ser  
 275 280 285  
 Gln Lys Leu Asn Phe Leu Glu Ser Gln Leu Pro Leu Val Lys Glu Asn  
 290 295 300  
 Leu Glu Lys Ala Glu Gln Ala Leu Ser Ala Tyr Arg Gln Gln His Gly  
 305 310 315 320  
 Ala Val Asp Ile Ser Ala Glu Ala Glu Ile Leu Leu Lys Gln Ala Ser  
 325 330 335  
 Glu Met Glu Thr Leu Ser Ile Gln Leu Lys Gln Lys Tyr Asp Glu Gln  
 340 345 350  
 Ser Gln Arg Leu Glu Ser Glu His Pro Asp Met Ile Ala Thr Asn Ala  
 355 360 365  
 Gln Ile Arg Arg Val Ser Asn Lys Leu Ala Ala Leu Glu Lys Arg Ile  
 370 375 380  
 Lys Asp Leu Pro Lys Thr Gln Gln Asn Met Val Ser Leu Ser Arg Asp  
 385 390 395 400  
 Val Gln Val Asn Thr Glu Leu Tyr Thr Ser Leu Leu Asn Ser Ala Gln  
 405 410 415  
 Glu Gln Arg Ile Ala Ala Ala Gly Ser Leu Gly Asn Ser Arg Ile Val  
 420 425 430  
 Asp Phe Ala Val Val Pro Glu Lys Pro Tyr Trp Pro Lys Pro Gly Leu  
 435 440 445  
 Leu Leu Ala Ile Ala Gly Leu Leu Gly Ile Ser Leu Gly Ser Ala Leu  
 450 455 460  
 Ile Phe Leu Arg His Ser Leu Gln Arg His Asp Asn Tyr Pro Ala Leu  
 465 470 475 480  
 Leu Glu Tyr Gln Val Gly Leu Pro Leu Phe Ala Ala Ile Pro His Ser  
 485 490 495  
 Lys Lys Gln Arg Arg Leu Ala Arg Leu Leu Asp Gln Gly Lys Glu Arg  
 500 505 510  
 Asp Thr Ala Ile Leu Val Ser His Asp Pro Leu Asp Ile Ser Val Glu  
 515 520 525  
 Ser Leu Arg Gly Leu Arg Thr Thr Leu Glu Ala Thr Leu Ala Ser Asp  
 530 535 540

Glu Ser Lys Val Ile Met Val Ser Ser Pro Ala Pro Gly Met Gly Lys  
 545 550 555 560  
 Ser Phe Ile Ser Thr Asn Leu Ala Ala Leu Leu Ala Ser Ile Arg Lys  
 565 570 575  
 Arg Val Leu Ile Ile Asp Ala Asp Met Arg Asn Gly Arg Leu His Glu  
 580 585 590  
 Thr Phe Ala Ile Ala Lys Gln Pro Gly Leu Ser Asp Leu Leu Ser Gly  
 595 600 605  
 Lys Val Ser Leu Gly Asp Val Ile Val Ser Leu Pro Glu Ile Gly Val  
 610 615 620  
 Asp Leu Ile Pro Arg Gly Glu Met Val Leu Asn Pro Ala Glu Leu Leu  
 625 630 635 640  
 Val Leu Gly Asp Leu Ala Asp Thr Leu Glu Gln Leu Lys Ser Phe Tyr  
 645 650 655  
 Asn His Ile Val Ile Asp Ser Pro Pro Ile Leu Gly Ala Thr Asp Ala  
 660 665 670  
 Ala Ile Met Gly Lys His Cys Asp Ala Thr Phe Leu Val Val Lys Glu  
 675 680 685  
 Gly Arg Tyr Thr Ala Gln Glu Leu Glu Val Ser Phe Arg Arg Leu Gln  
 690 695 700  
 Gln Val Gly Val Lys Pro Asn Gly Phe Ile Ile Asn Asp Met Lys Glu  
 705 710 715 720  
 Gly Ser Ser Tyr Tyr Pro Tyr Tyr Gly Tyr Ala Tyr Gln Arg Asp Asp  
 725 730 735  
 Met Arg Gln Lys Gln Thr Thr Ala Trp Gln Ala Arg Phe Gln Asn Leu  
 740 745 750  
 Asn Asp Trp Met Gly Arg Gln Asp Ala Glu Tyr Leu Pro Val Ala Asp  
 755 760 765  
 Asp Ala Glu Glu Leu His Asp Ser Ile Arg Ala  
 770 775

&lt;210&gt; 29

&lt;211&gt; 1416

&lt;212&gt; DNA

&lt;213&gt; Methylomonas 16a

&lt;400&gt; 29

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 ttgaggcaaa agaacttggt ggtgacgcc tgttggcgcc tggcgggaca gttattgtcg 120  
 gcaactggctt tgcttgcggt caccgcgcat ctgaccgaat tggtagcgcc ggcggttttc 180  
 gggcacgtgg cggtgctgaa tggcttcgtc gcgctggggg tggcgggtgt tgcctatccc 240  
 ttcattctgcg ccgggatgcg ttccaccaat gaatgccgaa atttccgcga gcgggcggca 300  
 ttgcatggat tgggtgttgc gctgacgacg cgatcgacgg cattggccat taccttgctg 360  
 ctgctgggag gcgcgctgta ttgctatatt gtcggtagtg aaatcggtt gttcgtgttg 420  
 accggattgc tgtagccgt caccgttcgc cgcgagttgg gcattcagct gatgataggc 480

```

gaacgcaagc aacgcggcgc cgcgctttgg caaaccagcg acagcatcct gcggccggtg 540
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gtctgtgcca gcgtgctggc caatacgtcg tggacgatcg taagcgatgc atggcaaaaa 660
aagcctaccg gcgatcgcgg cttcctgggg cggcaattcg agcgcggcct ttgggcttat 720
gccttgccgt tgatcccgat ggaattgatg ttctggctca acggcctggg cgaccgttac 780
gtgatcgggt atttcctaac ggcggtgaa gtgggggtgt acgcggccgc ttatacgtcg 840
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tttcaagcgg tttcccaagg caaaagcaaa gatgcatgtt cgctgctatg gctgtggata 960
ggggcggtcg tcgtgatgag tgttctgggc gtgacgctgg tctggttgtg caaggactgg 1020
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gccgcgggca cggccttgca tgccctgggc accgtgatgt ccagccgct gctggcgaga 1140
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ttcggcatcg aagcgtggt gttggccttg ctggccaagc cctggcgcaa gctccgcacg 1320
ggacggcagg cgcgatcgt tcaatccgaa gcggcgatgc ccgaaccga ctttgacgcc 1380
atcggagtga gagcggcggc gttctccaac gaatcc 1416

```

&lt;210&gt; 30

&lt;211&gt; 472

&lt;212&gt; PRT

&lt;213&gt; Methylobionas 16a

&lt;400&gt; 30

```

Met Leu Gly Lys Gly His Ser Asp Lys Ala Asn Leu Lys Glu Gly Phe
  1             5             10             15

```

```

Met Leu Asp Trp Leu Arg Gln Lys Asn Leu Leu Gly Asp Ala Cys Trp
          20          25          30

```

```

Ala Leu Ala Gly Gln Leu Leu Ser Ala Leu Ala Leu Leu Ala Gly Thr
    35          40          45

```

```

Arg Ile Leu Thr Glu Leu Val Thr Pro Ala Val Phe Gly His Val Ala
    50          55          60

```

```

Leu Leu Asn Gly Phe Val Ala Leu Gly Val Ala Val Phe Ala Tyr Pro
    65          70          75          80

```

```

Phe Ile Cys Ala Gly Met Arg Phe Thr Asn Glu Cys Arg Asn Phe Arg
          85          90          95

```

```

Glu Arg Ala Ala Leu His Gly Leu Val Phe Ala Leu Thr Thr Arg Ser
    100          105          110

```

```

Thr Ala Leu Ala Ile Thr Leu Leu Leu Leu Gly Gly Ala Leu Tyr Cys
    115          120          125

```

```

Tyr Phe Val Gly Ser Glu Ile Gly Leu Phe Val Leu Thr Gly Leu Leu
    130          135          140

```

```

Leu Ala Val Thr Val Arg Arg Glu Leu Gly Ile Gln Leu Met Ile Gly
    145          150          155          160

```

```

Glu Arg Lys Gln Arg Gly Ala Ala Leu Trp Gln Thr Ser Asp Ser Ile
    165          170          175

```

```

Leu Arg Pro Val Met ala Ile Trp Leu Val Trp Gly Leu Gly Gln Ser
    180          185          190

```

Pro Glu Ala Val Leu Leu Gly Tyr Val Cys Ala Ser Val Leu Ala Asn  
 195 200 205  
 Thr Leu Trp Thr Ile Val Ser Asp Ala Trp Gln Lys Lys Pro Thr Gly  
 210 215 220  
 Asp Arg Gly Phe Leu Gly Arg Gln Phe Glu Arg Gly Leu Trp Ala Tyr  
 225 230 235 240  
 Ala Leu Pro Leu Ile Pro Met Glu Leu Met Phe Trp Leu Asn Gly Leu  
 245 250 255  
 Gly Asp Arg Tyr Val Ile Gly Tyr Phe Leu Thr Ala Ala Glu Val Gly  
 260 265 270  
 Val Tyr Ala Ala Ala Tyr Thr Leu Val Asn Glu Ala Phe Asn Arg Ser  
 275 280 285  
 Ala Met Val Leu Leu Arg Thr Phe Gln Pro Ala Tyr Phe Gln Ala Val  
 290 295 300  
 Ser Gln Gly Lys Ser Lys Asp Ala Cys Ser Leu Leu Trp Leu Trp Ile  
 305 310 315 320  
 Gly Ala Val Val Val Met Ser Val Leu Gly Val Thr Leu Val Trp Leu  
 325 330 335  
 Cys Lys Asp Trp Leu Val Ala Gly Leu Leu Ala Glu Pro Tyr His Ala  
 340 345 350  
 Ala Gly Ala Leu Met Pro Val Ile Ala Ala Gly Thr Ala Leu His Ala  
 355 360 365  
 Leu Gly Thr Val Met Ser Gln Pro Leu Leu Ala Arg Lys Arg Thr Pro  
 370 375 380  
 Ile Leu Leu Arg Gly Arg Ile Cys Gly Ala Leu Ala Ala Leu Ile Thr  
 385 390 395 400  
 Leu Pro Leu Leu Val Ala His Phe Gly Leu Phe Gly Ala Ala Leu Ala  
 405 410 415  
 Asn Pro Val Tyr Phe Gly Ile Glu Ala Leu Val Leu Ala Leu Leu Ala  
 420 425 430  
 Lys Pro Trp Arg Lys Leu Arg Thr Gly Arg Gln Ala Arg Ile Val Gln  
 435 440 445  
 Ser Glu Ala Ala Met Pro Glu Pro Asp Phe Asp Ala Ile Gly Val Arg  
 450 455 460  
 Ala Ala Ala Phe Ser Asn Glu Ser  
 465 470

&lt;210&gt; 31

&lt;211&gt; 816

&lt;212&gt; DNA

&lt;213&gt; Methylomonas 16a

&lt;400&gt; 31

```

ccgataaaca ggtgtgaacc attgaacagc ttgaccatag tcattttgac gctgaacgag 60
gccgcccaatc tgccccggtg cctggcggcg attccgcaac gttaccctgt cgtgatcttg 120
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caagccaaag ccgctgcaca agcaagggga acagca 816

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&lt;210&gt; 32

&lt;211&gt; 272

&lt;212&gt; PRT

&lt;213&gt; Methylobionas 16a

&lt;400&gt; 32

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Pro Ile Asn Arg Cys Glu Pro Leu Asn Ser Leu Thr Ile Val Ile Leu
 1             5             10             15

Thr Leu Asn Glu Ala Ala Asn Leu Pro Arg Cys Leu Ala Ala Ile Pro
          20             25             30

Gln Arg Tyr Pro Val Val Ile Leu Asp Ser Gly Ser Ser Asp Asp Thr
          35             40             45

Leu Ser Ile Ala Glu Gly His Gly Cys Lys Ile Tyr Gln Asn Pro Trp
 50             55             60

Pro Gly Phe Ala Glu Gln Arg Asn Phe Ala Leu Asn Gln Cys Asp Ile
 65             70             75             80

Glu Thr Pro Trp Val Leu Phe Val Asp Ala Asp Glu Ile Tyr Pro Gln
          85             90             95

Val Phe Tyr Gln His Phe Asp Ser Gly Met Leu Gln Thr Gly Glu Ile
          100             105             110

Asp Val Leu Met Val Pro Ser Ile Leu Phe Leu Arg Gly Lys Arg Leu
          115             120             125

His His Ala Pro Gly Tyr Pro Ile Tyr His Pro Arg Leu Val Arg Arg
          130             135             140

Glu Thr Thr Arg Phe Val Arg Asn His Thr Gly His Gly Glu Ala Val
          145             150             155             160

Met Asp Ser Cys Arg Ile Gly Tyr Thr Asp Ile Pro Tyr Asp His Tyr
          165             170             175

Phe Tyr Asp Gly Glu Ile Ile Gln Trp Met His Lys His Val Asp Lys
          180             185             190

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Ala Ala Gln Glu Val Arg Leu Lys Pro Thr Gln Gly Ala Leu Met Thr  
 195 200 205

Thr Arg Gly Arg Leu Ser Val Met Leu Gly Arg Ser Trp Ser Arg Ile  
 210 215 220

Leu Ala Arg Phe Val Tyr His Tyr Leu Leu Arg Gly Gly Phe Leu Asp  
 225 230 235 240

Gly Ala Ala Gly Leu Glu Phe Thr Leu Met Phe Thr Trp Tyr Glu Ala  
 245 250 255

Ser Ile Tyr Leu Gln Ala Lys Ala Ala Ala Gln Ala Arg Gly Thr Ala  
 260 265 270

<210> 33

<211> 852

<212> DNA

<213> Methylomonas 16a

<400> 33

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 gacggcaaaa tcgatcggat tgccgagcaa tatagccaat gcctcgatct gaaacacgctc 180  
 aaggtgaatt tcaccggtaa tgcccagacc agggatcatg gcatcgctt ggcccagggc 240  
 gacatcatcg cctttccgga cgatgattgc gtgtatgaaa aggatgtgct ggaaaaagtg 300  
 gtaggcgaat ttgcatgccca gccaacgttg tcgattctgg tagccgggtc ctacgatttt 360  
 tccgcgaaac acttcagcat aggcgtcaac agccgtaaag cgcgttattt ttcccgttg 420  
 aacatgatgg ggggtggagt cagcgagttt tttgcgctgg cgcgtatcga caggcggcag 480  
 ttttatttgg accacgattt cggcatcggc tccaaatatg ccggggcgga aggttcgag 540  
 ttgctgtatc gcctgctgcg cgcgggcggg cgggcgttct acaagccgga tatcaaaatc 600  
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 ggtattggcg cctatatccg caaattcgcc aatcagcatg atccctatat cggctattac 720  
 atcctgcgca agatgctgat agccccgact ctgaaaatgc tgctggcctt gttgacgttc 780  
 aaccgggaa aactcgccta ttcgttttat aacctggtgg gcattatggcg cggatttttt 840  
 gcctatgggc gc 852

<210> 34

<211> 284

<212> PRT

<213> Methylomonas 16a

<400> 34

Met Lys Val Ser Leu Ile Leu Ala Thr Leu Gly Arg Asp Leu Glu Leu  
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Leu Asp Phe Leu Lys Ser Leu Leu Phe Gln Thr Tyr Lys Asn Phe Glu  
 20 25 30

Leu Ile Val Ile Asp Gln Asn Gln Asp Gly Lys Ile Asp Arg Ile Ala  
 35 40 45

Glu Gln Tyr Ser Gln Cys Leu Asp Leu Lys His Val Lys Val Asn Phe  
 50 55 60

Thr Gly Asn Ala Arg Ala Arg Asp His Gly Ile Ala Leu Ala Gln Gly  
 65 70 75 80



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<210> 35
<211> 1194
<212> DNA
<213> Methylobionas 16a
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tcgtcggatg	aatccatcga	tgggtgcttg	aaaccggcgg	acgtcaagct	gggcgaagc		180
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cqctatttcg	gctatctgca	gcgtgacgac	gtgatggcct	tgctggcgca	aagtcatttt		840

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 cggcgccatg ttcaacagca gctcgatccg gtcaaactgg cggagcgcgt ctggcaagca 1140  
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<210> 36

<211> 398

<212> PRT

<213> Methylomonas 16a

<400> 36

Met Glu Leu Gly Ile Val Thr Thr His Val Pro Pro Ala Lys Gly Tyr  
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Gly Gly Val Ser Val Thr Cys Gly Val Leu Thr Arg Ala Trp Ala Glu  
 20 25 30

Met Gly Leu Glu Met ala Leu Val Ser Ser Asp Glu Ser Ile Asp Gly  
 35 40 45

Cys Leu Lys Pro Ala Asp Val Lys Leu Gly Ala Ser Val Asp Val Asp  
 50 55 60

Leu Tyr Arg Cys Tyr Gly Phe Arg Arg Trp Gly Phe Gly Leu Gly Ala  
 65 70 75 80

Ile Pro Ser Leu Leu Arg Leu Cys Trp Gln Ala Pro Leu Val Tyr Ile  
 85 90 95

His Gly Val Ala Thr Trp Pro Ser Thr Leu Ala Ala Leu Phe Cys Cys  
 100 105 110

Leu Leu Arg Lys Pro Phe Met Val Ala Val His Gly Gly Leu Met Pro  
 115 120 125

Glu His Val Ala Leu Ile Lys Arg Lys Lys Arg His Lys Trp Trp Tyr  
 130 135 140

Tyr Lys Leu Leu Thr Phe Pro Thr Leu Arg Arg Ala Ile Ala Val His  
 145 150 155 160

Cys Thr Ser Asp Thr Glu Val Glu Gly Val Arg Asp Val Leu Gly Glu  
 165 170 175

Asn Ala Arg Val Leu Leu Val Pro Asn Gly Ile Asp Ser Arg Gly Val  
 180 185 190

Glu Glu Ala Pro Tyr Pro Ala Gly Glu Gly Met Gln Leu Cys Phe Leu  
 195 200 205

Gly His Val Gln Gln Glu Lys Gly Ile Asn Ala Phe Ile Arg Ala Trp  
 210 215 220

Leu Glu Val Arg Arg Pro Gly Asp Arg Leu Val Val Ala Gly Arg Ser  
 225 230 235 240

Val Asp Gly Asp Tyr Phe Ala Glu Phe Cys Ser Leu Val Glu Arg Ala  
 245 250 255

Asn Gly Ala Ile Arg Tyr Cys Gly Tyr Leu Gln Arg Asp Asp Val Met  
 260 265 270

Ala Leu Leu Ala Gln Ser His Phe Leu Val Leu Pro Ser Gly Leu Glu  
 275 280 285

Gln Val Gly Gly Met Arg Glu Asn Phe Gly Asn Val Val Ala Glu Ala  
 290 295 300

Leu Ala Ala Gly Arg Pro Val Leu Val Val Arg Gly Leu Ala Trp Asp  
 305 310 315 320

His Leu Pro Ala Leu Asn Ala Gly Leu Val Phe Asp Arg Asp Glu Ala  
 325 330 335

Ala Val Gln Ala Val Leu Arg Arg Ala Gln Ala Leu Asp Gln Ala Asp  
 340 345 350

Trp Leu Arg Met Ser Gln Ala Gly Arg Arg His Val Gln Gln Gln Leu  
 355 360 365

Asp Pro Val Lys Leu Ala Glu Arg Val Trp Gln Ala Met Thr Ala Ala  
 370 375 380

Val Pro Val Asp Glu Ala Lys Val Leu Ala Glu Glu Pro Lys  
 385 390 395

&lt;210&gt; 37

&lt;211&gt; 951

&lt;212&gt; DNA

&lt;213&gt; Methylobionas 16a

&lt;400&gt; 37

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gccaaaggcct cgttcaacca cggcggcact cgtcaatcgg gcgtcgatat gttggctcgac 240
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cagttgcgca ccttgcagga ccgcgaccgc ttccgcatca agaccgtgtt catttccaat 480
tcgttcgccc cctacagacg ttgcgccctg atgcaaatac gcggattccc ggctcacacc 540
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&lt;210&gt; 38

&lt;211&gt; 317

&lt;212&gt; PRT

&lt;213&gt; Methylobionas 16a

&lt;400&gt; 38

Met Thr His Lys Val Gly Leu Val Val Pro Thr Leu Asn Ala Gly Ala  
 1 5 10 15

Ser Trp Gln Gly Trp Leu Glu Ala Leu Ala Ala Gln Ser Arg Arg Pro  
 20 25 30

Asp Arg Leu Leu Leu Ile Asp Ser Ser Ser Ser Asp Asp Thr Val Ala  
 35 40 45

Leu Ala Arg Ala Arg Gly Phe Asp Ala His Val Ile Ala Lys Ala Ser  
 50 55 60

Phe Asn His Gly Gly Thr Arg Gln Ser Gly Val Asp Met Leu Val Asp  
 65 70 75 80

Met Asp Leu Ile Val Phe Leu Thr Gln Asp Ala Leu Leu Ala Asp Pro  
 85 90 95

Ser Ala Ile Glu Asn Leu Leu Gln Val Phe Val Asn Pro Gln Val Ala  
 100 105 110

Ala Ala Tyr Gly Arg Gln Leu Pro His Arg Asn Ala Gly Pro Ile Gly  
 115 120 125

Ala His Ala Arg Ile Phe Asn Tyr Pro Ala Gln Ser Gln Leu Arg Thr  
 130 135 140

Leu Gln Asp Arg Asp Arg Phe Gly Ile Lys Thr Val Phe Ile Ser Asn  
 145 150 155 160

Ser Phe Ala Ala Tyr Arg Arg Cys Ala Leu Met Gln Ile Gly Gly Phe  
 165 170 175

Pro Ala His Thr Ile Met Asn Glu Asp Thr Tyr Val Ala Gly Lys Met  
 180 185 190

Leu Leu Ser Gly Trp Ser Leu Ala Tyr Cys Ala Asp Ala Arg Val Phe  
 195 200 205

His Ser His Asp Tyr Ser Leu Leu Glu Glu Phe Arg Arg Tyr Phe Asp  
 210 215 220

Ile Gly Val Phe His Ala Gln Asn Pro Trp Leu Gln Gln Thr Phe Gly  
 225 230 235 240

Gly Ala Ser Gly Glu Gly Ala Arg Phe Val Leu Ser Glu Met Arg Tyr  
 245 250 255

Leu Ser Asn Thr Ala Pro Trp Leu Met Phe Ser Ala Phe Leu Arg Thr  
 260 265 270

Gly Leu Lys Trp Ala Gly Tyr Lys Leu Gly Gly Leu His Arg Gly Trp  
 275 280 285

Pro Leu Ala Leu Ser Arg Arg Leu Ser Leu His Lys Gly Tyr Trp Val  
 290 295 300

Ala Thr Glu Arg Glu Tyr Pro Asn Met Pro Gly Cys Arg  
 305 310 315

<210> 39  
 <211> 1170  
 <212> DNA  
 <213> *Methylobacterium* 16a

<220>  
 <223> ORF1

<220>  
 <223> nirF gene

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 gacctgcgcg ccaccggcga tttgggtgtc gtgatcgagc gcgagaccgg cagtgtgcaa 120  
 gtcacaaaca ccagcacgcc caagatgctg agccgcacgc aaggcctggg cgatttgtct 180  
 cagcgttcgg tgggtgtctc gcgtgatcag cgctatgcct atgtattcgg tcgcgacggc 240  
 ggcttgagca aaatcgatct gttgcaggac aaaatcgaaa aacgcgtcgt gcaagccggg 300  
 aacagcatag gcggggcgat ttcccaggat ggcaaagtca tcgccgtatc caactatacg 360  
 ccggggcgcg tcaagctgtt cgatgccgag accttggagc agttggccga gattccggcc 420  
 gtttacggcg acgacaacca gttatccaaa gtggtcggct tggtcgatgc accggggcgg 480  
 cgtttcggtt gcagcctgtt cgaaggtaac gagatttggc tgatagacgc caagaatcca 540  
 cgccagccgg tcgtcaagaa attcaaggac atcggcaagc ggccttatga tgccttgctg 600  
 acgccggatg gccatttcta cgccggccga ctgttcggcg aaaaaggcct ggctttgctg 660  
 gatttatggc agccggagct aggcgtcaaa cacatcctgg aagactacgg caaggacgac 720  
 gagcaattgc cggtttaca aatgccgcat ctggaaggct ggacggtagc cggtgatctg 780  
 ctgttcgtgc cggccatcgg cctgcatgag gtgttggtga tcgataaaca cgattgggag 840  
 ctgttcaaac gcattccggt cgtcggacaa cccgtgttcg tgatgtcccg accggatggt 900  
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 aaggatttca atatcgtcaa gaccctgcaa ccgggtaagg ccgtgctgca catggagttc 1020  
 agcccgcgcg gcgaagccgt ctggatggcg gtgcgcgacg aggacagggt aatgggttac 1080  
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 ttcagtaatc gcgccaatca gttggggcgt 1170

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 <211> 390  
 <212> PRT  
 <213> *Methylobacterium* 16a

<220>  
 <223> NirF

<400> 40  
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 20 25 30  
 Glu Arg Glu Thr Gly Ser Val Gln Val Ile Asn Thr Ser Thr Pro Lys  
 35 40 45  
 Met Leu Ser Arg Ile Glu Gly Leu Gly Asp Leu Ser His Ala Ser Val  
 50 55 60  
 Val Phe Ser Arg Asp Gln Arg Tyr Ala Tyr Val Phe Gly Arg Asp Gly  
 65 70 75 80  
 Gly Leu Ser Lys Ile Asp Leu Leu Gln Asp Lys Ile Glu Lys Arg Val  
 85 90 95

Val Gln Ala Gly Asn Ser Ile Gly Gly Ala Ile Ser Gln Asp Gly Lys  
 100 105 110  
 Val Ile Ala Val Ser Asn Tyr Thr Pro Gly Gly Val Lys Leu Phe Asp  
 115 120 125  
 Ala Glu Thr Leu Glu Gln Leu Ala Glu Ile Pro Ala Val Tyr Gly Asp  
 130 135 140  
 Asp Asn Gln Leu Ser Lys Val Val Gly Leu Val Asp Ala Pro Gly Gly  
 145 150 155 160  
 Arg Phe Val Cys Ser Leu Phe Glu Gly Asn Glu Ile Trp Leu Ile Asp  
 165 170 175  
 Ala Lys Asn Pro Arg Gln Pro Val Val Lys Lys Phe Lys Asp Ile Gly  
 180 185 190  
 Lys Arg Pro Tyr Asp Ala Leu Leu Thr Pro Asp Gly His Phe Tyr Ala  
 195 200 205  
 Ala Gly Leu Phe Gly Glu Lys Gly Leu Ala Leu Leu Asp Leu Trp Gln  
 210 215 220  
 Pro Glu Leu Gly Val Lys His Ile Leu Glu Asp Tyr Gly Lys Asp Asp  
 225 230 235 240  
 Glu Gln Leu Pro Val Tyr Lys Met Pro His Leu Glu Gly Trp Thr Val  
 245 250 255  
 Ala Gly Asp Leu Leu Phe Val Pro Ala Ile Gly Leu His Glu Val Leu  
 260 265 270  
 Val Ile Asp Lys His Asp Trp Glu Leu Val Lys Arg Ile Pro Val Val  
 275 280 285  
 Gly Gln Pro Val Phe Val Met Ser Arg Pro Asp Gly Arg Gln Val Trp  
 290 295 300  
 Val Asn Phe Ala Phe Pro Asp Asn Gln Thr Val Gln Val Ile Asp Val  
 305 310 315 320  
 Lys Asp Phe Asn Ile Val Lys Thr Leu Gln Pro Gly Lys Ala Val Leu  
 325 330 335  
 His Met Glu Phe Ser Pro Arg Gly Glu Ala Val Trp Met ala Val Arg  
 340 345 350  
 Asp Glu Asp Arg Val Met Val Tyr Asp Thr Asp Ser Phe Asn Glu Thr  
 355 360 365  
 Ala Arg Leu Pro Ala Gln Lys Pro Ser Gly Ile Phe Phe Ser Asn Arg  
 370 375 380  
 Ala Asn Gln Leu Gly Leu  
 385 390

<210> 41  
 <211> 453

<212> DNA  
 <213> *Methylobacterium* 16a

<220>  
 <223> ORF2

<220>  
 <223> nirD gene

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 gcggtttcagg tggtggcoga gcagcaaatg atcagccgca tcggccccgt gatcgcgccg 180  
 aacgccatcg gcaatagcgc cttggtggcg atggcggtgc cggagcagga tttggccccgt 240  
 gtcgccccct tggtagcgc ctatccgga gtcaatcata actatgagcg ggaaaaccgc 300  
 ttcaatttgt ggtttgtgct gatcgctcc gatcatactc acttgagcg ggtgattgcc 360  
 gatatcgaga ctcaaaccgg ttatcaagcc atgctgttgc cgatgctggc cgattatttc 420  
 atcaacctgg gttttgaact caatctgaac gac 453

<210> 42  
 <211> 151  
 <212> PRT  
 <213> *Methylobacterium* 16a

<220>  
 <223> NirD

<400> 42  
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 Val Thr Glu Gly Glu Val Leu Ala Ala Phe Gln Val Leu Ala Glu Gln  
 35 40 45  
 Gln Met Ile Ser Arg Ile Gly Pro Val Ile Ala Pro Asn Ala Ile Gly  
 50 55 60  
 Asn Ser Ala Leu Val Ala Met ala Val Pro Glu Gln Asp Leu Ala Arg  
 65 70 75 80  
 Val Ala Ala Leu Val Ser Ala Tyr Pro Glu Val Asn His Asn Tyr Glu  
 85 90 95  
 Arg Glu Asn Arg Phe Asn Leu Trp Phe Val Leu Ile Ala Ser Asp His  
 100 105 110  
 Thr His Leu Gln Arg Val Ile Ala Asp Ile Glu Thr Gln Thr Gly Tyr  
 115 120 125  
 Gln Ala Met Leu Leu Pro Met Leu Ala Asp Tyr Phe Ile Asn Leu Gly  
 130 135 140  
 Phe Glu Leu Asn Leu Asn Asp  
 145 150

<210> 43  
 <211> 504

<212> DNA  
 <213> *Methylomonas* 16a

<220>  
 <223> ORF3

<220>  
 <223> nirL gene

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 ctgggacgtc tgaaaacgga aggtttgatc aggcgctggg gcgtcgtggt caagcaccgg 180  
 caactaggtt atcgcgcgcaa tgcgatgac gtgatggata ttcctgatga tcaagttgcg 240  
 gaaatgggccc gccgtgtcag ccagcacagc ttcgtcaatc tgtgttatcg ccgaccacgt 300  
 caaggcgagg tttggccgta taacctttat tgcagtatac acggcaaaaa tcgcgagacg 360  
 gttttgcagc aatgggcccga tctgcaacaa agttgcggcc tggaagcctg tcggcacgag 420  
 attttattca gtcgtcgttg tttcaagcag cgtggggcta tttataaagc gcccggtgatt 480  
 gagccattgg agtttagtca tgga 504

<210> 44  
 <211> 168  
 <212> PRT  
 <213> *Methylomonas* 16a

<220>  
 <223> NirL

<400> 44  
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 Asp Glu Gln Asp Val Ile Ala Arg Leu Gly Arg Leu Lys Thr Glu Gly  
 35 40 45  
 Leu Ile Arg Arg Trp Gly Val Val Val Lys His Arg Gln Leu Gly Tyr  
 50 55 60  
 Arg Ala Asn Ala Met Ile Val Met Asp Ile Pro Asp Asp Gln Val Ala  
 65 70 75 80  
 Glu Met Gly Arg Arg Val Ser Gln His Ser Phe Val Asn Leu Cys Tyr  
 85 90 95  
 Arg Arg Pro Arg Gln Gly Glu Val Trp Pro Tyr Asn Leu Tyr Cys Met  
 100 105 110  
 Ile His Gly Lys Asn Arg Glu Thr Val Leu Gln Gln Trp Ala Asp Leu  
 115 120 125  
 Gln Gln Ser Cys Gly Leu Glu Ala Cys Arg His Glu Ile Leu Phe Ser  
 130 135 140  
 Arg Arg Cys Phe Lys Gln Arg Gly Ala Ile Tyr Lys Ala Pro Val Ile  
 145 150 155 160



Glu Pro Leu Glu Phe Ser His Gly  
165

<210> 45  
<211> 441  
<212> DNA  
<213> *Methylobacterium* 16a

<220>  
<223> ORF4

<220>  
<223> nirG gene

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tcgccttata gatatgtgc cgagcagctt ggtgtggccg aggcggaatt gctggagagg 120  
ctgcaaacct tggtgaacca gggcgtttta tcgcgctttg ggccgatgta tcacgccgag 180  
caaatgggcg gcgccttgac cttggcgcg atgaagggtc caggggagcg ttctgcagaa 240  
attgcaggca tcgtcaacgg ctttccggag gtggcgcata actatgcgcg taaccacgcc 300  
ttgaacatgt ggtttgtgtt ggcgaccgaa aagccggaac aagtgcaggc ggtcatcgat 360  
gccatcgaac ggcaactgg cttgacggtc tataacatgc cgaaaatcaa ggaatattac 420  
gtgggcttgc aactggaggc c 441

<210> 46  
<211> 147  
<212> PRT  
<213> *Methylobacterium* 16a

<220>  
<223> NirG

<400> 46  
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Pro Ile Cys Glu Ser Pro Tyr Arg Tyr Val Ala Glu Gln Leu Gly Val  
20 25 30  
Ala Glu Ala Glu Leu Leu Glu Arg Leu Gln Thr Leu Leu Asn Gln Gly  
35 40 45  
Val Leu Ser Arg Phe Gly Pro Met Tyr His Ala Glu Gln Met Gly Gly  
50 55 60  
Ala Leu Thr Leu Ala Ala Met Lys Val Pro Gly Glu Arg Phe Asp Glu  
65 70 75 80  
Ile Ala Gly Ile Val Asn Gly Phe Pro Glu Val Ala His Asn Tyr Ala  
85 90 95  
Arg Asn His Ala Leu Asn Met Trp Phe Val Leu Ala Thr Glu Lys Pro  
100 105 110  
Glu Gln Val Gln Ala Val Ile Asp Ala Ile Glu Arg Gln Thr Gly Leu  
115 120 125  
Thr Val Tyr Asn Met Pro Lys Ile Lys Glu Tyr Tyr Val Gly Leu Gln  
130 135 140

Leu Glu Ala  
145

<210> 47  
<211> 498  
<212> DNA  
<213> *Methylobionas* 16a

<220>  
<223> ORF5

<220>  
<223> nirH gene

<400> 47  
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acccaggccg gcttgccgct ggctgcggaa ccttatcagg ccgtcgccga gcaattgggc 120  
atcacggctc aagaattgat gctgcgcatg gccgatatgc tggaagctgg catcattcgg 180  
cggattgcgg cggtgccgaa tcattacaaa ctgggttatc gtcataacgg catgacgggc 240  
tggtgatgct atgaccggca tgctgcacagc ctggggcagc gcgtcgccga attgccgttc 300  
gtcagtcatt gctaccaacg gcctcgccat ttgccggatt ggccgtataa cctgttcgcg 360  
atggtgcatg gcaagacgga acaagacgcc gaaaaacaaa ttgccgtgat cgccgaattg 420  
ttgggagagg attgctaccg gcacgcgggt ctgtacagca ccaagatttt gaagaaaacc 480  
ggcttgagga ttgcgggg 498

<210> 48  
<211> 166  
<212> PRT  
<213> *Methylobionas* 16a

<220>  
<223> NirH

<400> 48  
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1 5 10 15  
Ile Ile Gln Ala Thr Gln Ala Gly Leu Pro Leu Val Ala Glu Pro Tyr  
20 25 30  
Gln Ala Val Ala Glu Gln Leu Gly Ile Thr Ala Gln Glu Leu Met Leu  
35 40 45  
Arg Met ala Asp Met Leu Glu Ala Gly Ile Ile Arg Arg Ile Ala Ala  
50 55 60  
Val Pro Asn His Tyr Lys Leu Gly Tyr Arg His Asn Gly Met Thr Val  
65 70 75 80  
Trp Asp Val Asp Asp Arg His Val Asp Ser Leu Gly Gln Arg Val Ala  
85 90 95  
Glu Leu Pro Phe Val Ser His Cys Tyr Gln Arg Pro Arg His Leu Pro  
100 105 110  
Asp Trp Pro Tyr Asn Leu Phe Ala Met Val His Gly Lys Thr Glu Gln  
115 120 125  
Asp Ala Glu Lys Gln Ile Ala Val Ile Ala Glu Leu Leu Gly Glu Asp  
130 135 140

Cys Tyr Arg His Ala Val Leu Tyr Ser Thr Lys Ile Leu Lys Lys Thr  
 145 150 155 160

Gly Leu Arg Ile Ala Gly  
 165

<210> 49  
 <211> 1137  
 <212> DNA  
 <213> *Methylobacter* 16a

<220>  
 <223> ORF6

<220>  
 <223> nirJ gene

<400> 49  
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 cgcaaacggt ccggcccggg ggtaattctgg aatctgatcc gtctgtgcaa cctgacttgc 120  
 aagcattgct ataccacgtc cgccgacatc gattttccgg gtgaactgac gacgccggaa 180  
 atttatgctg tgatggacga tttgaaagcc ttcaagggtc cggtattgat tctgtccggc 240  
 ggagagccgt tgctgcatcc ggatattttt ccgatttcgc aacgcgccag cgacatgggc 300  
 ttttacgtgg ccttgtccag caacggcacg ctgctcgaca aaaacaatat cgagcaaadc 360  
 gccgcgatcg attatcaata tattggcgct agtctggacg gcatgcgcga ggcgcacgac 420  
 aagttccgcc agaagcaagg ctctttcgac gcctcgctgg ccggcatccg tttatgccgc 480  
 gagcatggca tcaaggcccg cgtgcgcttc acgttgacgc gggacaacgc tcacgatttc 540  
 gatgccttgc tgcagttgat ggacgaggag gacatcgaca aattctatct gtcgcatctg 600  
 aattacggcg gccgcggcaa taaaaaccgg aaagacgatg ccgagtttca gttgacccgc 660  
 aaggtcatgg acgccttggt cgaaaaggcg ctgagctggg aacagcaagg cctacaccgc 720  
 gaagtgggtca ccggcaacaa cgatgccgat gccgtatatt tcttgcattg ggtcaaacgc 780  
 cgctttcccg agcgcgccga gcatatccag gccaaagtgc agcaatgggg ccgcaatgct 840  
 tccggcgta caagtagcaa tatcgataat ctgggtaacg tgcatcccga taccttttgg 900  
 tggcattaca acttgggcag tgtccgccag ccggcgtttt ccgagatatg gcaggatgtg 960  
 tccgacccat tgatggcccg gctgaaggcc tcgccgcgcc cgctgaaagg ccgctgcggc 1020  
 acctgtcatt atcaaagcat ttgcaacggc aatacccgcg tccgcgccca acaactgacc 1080  
 ggcgattttt gggctgaaga tccaggctgc tacctggatg acgaggaagt tttcagc 1137

<210> 50  
 <211> 379  
 <212> PRT  
 <213> *Methylobacter* 16a

<220>  
 <223> Nir J

<400> 50  
 Met Phe Arg Leu Ser Gln Tyr Met Arg Glu Leu Val His Ser Thr Pro  
 1 5 10 15  
 Leu Gly Lys Pro Arg Lys Pro Ser Gly Pro Val Val Ile Trp Asn Leu  
 20 25 30  
 Ile Arg Arg Cys Asn Leu Thr Cys Lys His Cys Tyr Thr Thr Ser Ala  
 35 40 45  
 Asp Ile Asp Phe Pro Gly Glu Leu Thr Thr Pro Glu Ile Tyr Ala Val  
 50 55 60

Met Asp Asp Leu Lys Ala Phe Lys Val Pro Val Leu Ile Leu Ser Gly  
 65 70 75 80  
 Gly Glu Pro Leu Leu His Pro Asp Ile Phe Pro Ile Ser Gln Arg Ala  
 85 90 95  
 Ser Asp Met Gly Phe Tyr Val Ala Leu Ser Ser Asn Gly Thr Leu Ile  
 100 105 110  
 Asp Lys Asn Asn Ile Glu Gln Ile Ala Ala Ile Asp Tyr Gln Tyr Ile  
 115 120 125  
 Gly Val Ser Leu Asp Gly Met Arg Glu Ala His Asp Lys Phe Arg Gln  
 130 135 140  
 Lys Gln Gly Ser Phe Asp Ala Ser Leu Ala Gly Ile Arg Leu Cys Arg  
 145 150 155 160  
 Glu His Gly Ile Lys Ala Gly Val Arg Phe Thr Leu Thr Arg Asp Asn  
 165 170 175  
 Ala His Asp Phe Asp Ala Leu Leu Gln Leu Met Asp Glu Glu Asp Ile  
 180 185 190  
 Asp Lys Phe Tyr Leu Ser His Leu Asn Tyr Gly Gly Arg Gly Asn Lys  
 195 200 205  
 Asn Arg Lys Asp Asp Ala Glu Phe Gln Leu Thr Arg Lys Val Met Asp  
 210 215 220  
 Ala Leu Phe Glu Lys Ala Leu Ser Trp Glu Gln Gln Gly Leu His Arg  
 225 230 235 240  
 Glu Val Val Thr Gly Asn Asn Asp Ala Asp Ala Val Tyr Phe Leu His  
 245 250 255  
 Trp Val Lys Arg Arg Phe Pro Glu Arg Ala Glu His Ile Gln Ala Lys  
 260 265 270  
 Leu Gln Gln Trp Gly Gly Asn Ala Ser Gly Val Asn Val Ala Asn Ile  
 275 280 285  
 Asp Asn Leu Gly Asn Val His Pro Asp Thr Phe Trp Trp His Tyr Asn  
 290 295 300  
 Leu Gly Ser Val Arg Gln Arg Pro Phe Ser Glu Ile Trp Gln Asp Val  
 305 310 315 320  
 Ser Asp Pro Leu Met ala Gly Leu Lys Ala Ser Pro Arg Pro Leu Lys  
 325 330 335  
 Gly Arg Cys Gly Thr Cys His Tyr Gln Ser Ile Cys Asn Gly Asn Thr  
 340 345 350  
 Arg Val Arg Ala Gln Gln Leu Thr Gly Asp Phe Trp Ala Glu Asp Pro  
 355 360 365  
 Gly Cys Tyr Leu Asp Asp Glu Glu Val Phe Ser  
 370 375

<210> 51  
 <211> 2760  
 <212> DNA  
 <213> *Methylobionas* 16a

<220>  
 <223> ORF7

<220>  
 <223> nasA gene

<400> 51  
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 gccagggtgc tcgatgccga aaaccatgtc gtcaatattg ccggcgatcc acagcatcag 120  
 tccaatttcg gccgactgtg ctccaagggc gggcgctgg gtgataccgt cggctctggaa 180  
 ggccgccttt tatacccgga aatcgatggc cggcgctgg attggccac ggtgctggac 240  
 cggatcgcgg ctaaatcaa tgcgatcatt gccgagcacg gcccggacgc ggtggcgttt 300  
 tatgtgtccg gacagtgtt gaccgaggat tattatgtcg ccaacaaatt gatgaagggc 360  
 ttcacgggt cggcgaatat cgataccaat tccaggctgt gcatgtcctc ggcggtagtc 420  
 ggttacaagc gtgcgttcgg cggcgatgcg gtgccctgta atttcgagga tctggaacgg 480  
 gcagacttga tcgtgctggt cggttccaac ggggcctggt gccatccgat tgcgtttcag 540  
 cgcgtgcgtc aggccaaagat agacaatccg gcgctgaaaa tcgtactaat agaccgcgt 600  
 caaaccagca gtcgcgatat cgccgatcgg catctggcca tcaagcccg catggacggc 660  
 ttgttggtca atggcttgct gggttatctg gccgaaaccg gcgcttgga tcaggattac 720  
 atcgaacgac actgtgaagg ttttgccgag gccttgccga cggctcgagc gagcgccgc 780  
 gattttaccg ttctggccaa ccgttgccgc gtggcgccgc acgatctggc gcaactgttt 840  
 gcctggtttg ccggttgga caaggtcgtg accgtttatt cgcaaggcat caaccagtc 900  
 agttcaggct ctgataaatg caacgccatc atcaattgcc atctggccag cggcaaaatc 960  
 ggcaaacccg gctgcggacc gttctcgttt accggccagc ccaacgcgat gggcgggcgc 1020  
 gaggtcggtg ggctggcgaa catgctggcc gcgcacatgg atttgaaaa tccagcgcat 1080  
 gtcgatagaa tcgcgcggtt ttggcaaac ccgcgctcg cccgcaaac aggcctgaaa 1140  
 gcggtagaaa ttttcgacgc catcgccgac ggtcgcatca aggccttatg gatcatggcg 1200  
 accaaccocg tggatcgcg gccggatgcc gacaaggtaa tcgaagcact taagcaatgc 1260  
 gaatttttgc tggatcgcga ttgcacgcc aacaccgaca ccgtggagct ggcgcagtc 1320  
 aaactgccgg ccaccggctg gagcgagaag gacggcaccg tcaccaatct ggaacgtgc 1380  
 atctcgccgc agcggccatt attccagcct tcggcgagcg cgaaccggga ttggtggatc 1440  
 gtcagccagg ttgctaagcg catggggttt gccggtctcg attatcgaaa cagcgccgaa 1500  
 atcttcaagg aacacgcggc cttgtccggt tttgaaaatg atgcagcgca ggggggcagg 1560  
 gattttgata tttcaggcct ggcaacgctg gatcaggccc agtttgacgc cttagtccg 1620  
 atacaatggc ctgtcacagg caagactcaa ggcggaacgg cgcgcctgtt cgaagacggt 1680  
 cgttttttta ccgacaccgg caaggccaga ttcatcgac tcgagcccg ctcgccaatg 1740  
 cacgccccca caccggatta tccgctggct ttgaataccg gccgcaccc cgatcaatgg 1800  
 cacacgatga ccgcaccgc gctgtccgcg aagctcaatc aacacaagcc ggaaccgttc 1860  
 gtggagattc atccgcagga tgcgttgctg tgggggctca aagcaaacgc cctggcccg 1920  
 atcgaaagcc gttggggcg cgtgttgccg cgggtcgacg tcagcgaggg tcagcaacc 1980  
 ggcagcgtgt tcgtgcccat gcaactggac gccagctca gcagtcatgg ccgagtcggc 2040  
 gccgtggtca accctgtcgt ggaccctttg tccgggcaac cggaaagcaa gcaaaccccg 2100  
 gtgcgcatcg cggcttgggc accttgctgg caagcgatgg tattgacgaa aatgccattg 2160  
 gacatcgacg attgcaata ccacgtcaaa ataaggggccc atggcttttg gcgctatcat 2220  
 ttggcggtac aatcccagcg gccagacttg ccggcggtgg gccgcggcat tgtcggcagg 2280  
 ggggcggcca aaccatga ttgcgtggaa tatctcgacc tggccgctgg cgattaccgc 2340  
 tttgccgaga tgcgggatca aacccttcat gcctgcattg tcattactca taatggggag 2400  
 ttgccggacc ctggctggct ggccagccta ttcggcaaac cgagattgac ccgcaaggaa 2460  
 cgcttcaacc tgcacgcgg cgtgcgcgg caaggggaaa tcgatagcgg caaacgatc 2520  
 tgctcctgct tcaacgtcgg cgaaaaaac atcgtgcaag ccattcaaac ccgacatttg 2580  
 agctgtgtaa cagatatagg caactgcctg catgcgggaa cgggttggtg ttcgtgttta 2640  
 ccggaattaa aaagcatttt ggccacgcg aaaacgatcg atcctgcctc gctgccatt 2700  
 cagccaactc aaatcccgcg ggcacggag ggggaaggag aagccttttt ttcaggtcaa 2760

<210> 52  
 <211> 920  
 <212> PRT  
 <213> *Methylobionas* 16a

<220>  
 <223> NasA

<400> 52  
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 Cys Gly Ile Glu Ala Arg Val Leu Asp Ala Glu Asn His Val Val Asn  
                     20                    25                    30  
 Ile Ala Gly Asp Pro Gln His Gln Ser Asn Phe Gly Arg Leu Cys Ser  
                     35                    40                    45  
 Lys Gly Ala Ala Leu Gly Asp Thr Val Gly Leu Glu Gly Arg Leu Leu  
                     50                    55                    60  
 Tyr Pro Glu Ile Asp Gly Arg Arg Val Asp Trp Pro Thr Val Leu Asp  
     65                    70                    75                    80  
 Arg Ile Ala Ala Lys Phe Asn Ala Ile Ile Ala Glu His Gly Pro Asp  
                     85                    90                    95  
 Ala Val Ala Phe Tyr Val Ser Gly Gln Leu Leu Thr Glu Asp Tyr Tyr  
                     100                    105                    110  
 Val Ala Asn Lys Leu Met Lys Gly Phe Ile Gly Ser Ala Asn Ile Asp  
                     115                    120                    125  
 Thr Asn Ser Arg Leu Cys Met Ser Ser Ala Val Val Gly Tyr Lys Arg  
     130                    135                    140  
 Ala Phe Gly Ala Asp Ala Val Pro Cys Asn Phe Glu Asp Leu Glu Arg  
     145                    150                    155                    160  
 Ala Asp Leu Ile Val Leu Val Gly Ser Asn Ala Ala Trp Cys His Pro  
                     165                    170                    175  
 Ile Ala Phe Gln Arg Met Arg Gln Ala Lys Ile Asp Asn Pro Ala Leu  
                     180                    185                    190  
 Lys Ile Val Leu Ile Asp Pro Arg Gln Thr Ser Ser Cys Asp Ile Ala  
                     195                    200                    205  
 Asp Arg His Leu Ala Ile Lys Pro Gly Met Asp Gly Leu Leu Phe Asn  
     210                    215                    220  
 Gly Leu Leu Val Tyr Leu Ala Glu Thr Gly Ala Leu Asp Gln Asp Tyr  
     225                    230                    235                    240  
 Ile Glu Arg His Cys Glu Gly Phe Ala Glu Ala Leu Ala Thr Ala Arg  
                     245                    250                    255  
 Ala Ser Ala Ala Asp Phe Thr Val Leu Ala Asn Arg Cys Gly Val Ala  
                     260                    265                    270

Ala His Asp Leu Ala Gln Leu Phe Ala Trp Phe Ala Gly Leu Asp Lys  
 275 280 285  
 Val Val Thr Val Tyr Ser Gln Gly Ile Asn Gln Ser Ser Ser Gly Ser  
 290 295 300  
 Asp Lys Cys Asn Ala Ile Ile Asn Cys His Leu Ala Ser Gly Lys Ile  
 305 310 315 320  
 Gly Lys Pro Gly Cys Gly Pro Phe Ser Phe Thr Gly Gln Pro Asn Ala  
 325 330 335  
 Met Gly Gly Arg Glu Val Gly Gly Leu Ala Asn Met Leu Ala Ala His  
 340 345 350  
 Met Asp Leu Glu Asn Pro Ala His Val Asp Arg Val Ala Arg Phe Trp  
 355 360 365  
 Gln Thr Asp Ser Val Ala Arg Lys Pro Gly Leu Lys Ala Val Glu Ile  
 370 375 380  
 Phe Asp Ala Ile Ala Asp Gly Arg Ile Lys Ala Leu Trp Ile Met ala  
 385 390 395 400  
 Thr Asn Pro Val Val Ser Met Pro Asp Ala Asp Lys Val Ile Glu Ala  
 405 410 415  
 Leu Lys Gln Cys Glu Phe Leu Leu Val Ser Asp Cys Ile Ala Asn Thr  
 420 425 430  
 Asp Thr Val Glu Leu Ala His Val Lys Leu Pro Ala Thr Gly Trp Ser  
 435 440 445  
 Glu Lys Asp Gly Thr Val Thr Asn Leu Glu Arg Arg Ile Ser Arg Gln  
 450 455 460  
 Arg Pro Leu Phe Gln Pro Ser Gly Glu Ala Lys Pro Asp Trp Trp Ile  
 465 470 475 480  
 Val Ser Gln Val Ala Lys Arg Met Gly Phe Ala Gly Phe Asp Tyr Arg  
 485 490 495  
 Asn Ser Ala Glu Ile Phe Lys Glu His Ala Ala Leu Ser Gly Phe Glu  
 500 505 510  
 Asn Asp Ala Ala Gln Gly Gly Arg Asp Phe Asp Ile Ser Gly Leu Ala  
 515 520 525  
 Thr Leu Asp Gln Ala Gln Phe Asp Ala Leu Val Pro Ile Gln Trp Pro  
 530 535 540  
 Val Thr Gly Lys Thr Gln Gly Gly Thr Ala Arg Leu Phe Glu Asp Gly  
 545 550 555 560  
 Arg Phe Phe Thr Asp Thr Gly Lys Ala Arg Phe Ile Ala Leu Glu Pro  
 565 570 575  
 Arg Ser Pro Met His Ala Pro Thr Pro Asp Tyr Pro Leu Val Leu Asn  
 580 585 590

Thr Gly Arg Ile Arg Asp Gln Trp His Thr Met Thr Arg Thr Ala Leu  
 595 600 605  
 Ser Ala Lys Leu Asn Gln His Lys Pro Glu Pro Phe Val Glu Ile His  
 610 615 620  
 Pro Gln Asp Ala Leu Arg Trp Gly Leu Lys Ala Asn Ala Leu Ala Arg  
 625 630 635 640  
 Ile Glu Ser Arg Trp Gly Gly Met Leu Ala Arg Val Asp Val Ser Glu  
 645 650 655  
 Ala Gln Gln Pro Gly Ser Val Phe Val Pro Met His Trp Thr Ala Gln  
 660 665 670  
 Leu Ser Ser His Gly Arg Val Gly Ala Val Val Asn Pro Val Val Asp  
 675 680 685  
 Pro Leu Ser Gly Gln Pro Glu Ser Lys Gln Thr Pro Val Arg Ile Ala  
 690 695 700  
 Ala Trp Ala Pro Cys Trp Gln Ala Met Val Leu Thr Lys Met Pro Leu  
 705 710 715 720  
 Asp Ile Asp Asp Cys Glu Tyr His Val Lys Ile Arg Gly His Gly Phe  
 725 730 735  
 Trp Arg Tyr His Leu Ala Asp Gln Ser Gln Arg Pro Asp Leu Pro Ala  
 740 745 750  
 Trp Gly Arg Gly Ile Val Gly Arg Gly Ala Ala Lys Pro Asn Asp Cys  
 755 760 765  
 Val Glu Tyr Leu Asp Leu Ala Ala Gly Asp Tyr Arg Phe Ala Glu Met  
 770 775 780  
 Arg Asp Gln Thr Leu His Ala Cys Met Phe Ile Thr His Asn Gly Glu  
 785 790 795 800  
 Leu Pro Asp Pro Gly Trp Leu Ala Ser Leu Phe Gly Lys Pro Arg Leu  
 805 810 815  
 Thr Arg Lys Glu Arg Phe Asn Leu Leu Ser Gly Val Pro Pro Gln Gly  
 820 825 830  
 Glu Ile Asp Ser Gly Lys Thr Ile Cys Ser Cys Phe Asn Val Gly Glu  
 835 840 845  
 Lys Thr Ile Val Gln Ala Ile Gln Thr Arg His Leu Ser Cys Val Thr  
 850 855 860  
 Asp Ile Gly Asn Cys Leu His Ala Gly Thr Gly Cys Gly Ser Cys Leu  
 865 870 875 880  
 Pro Glu Leu Lys Ser Ile Leu Ala His Ala Lys Thr Ile Asp Pro Ala  
 885 890 895  
 Ser Leu Pro Ile Gln Pro Thr Gln Ile Pro Pro Ala Ser Glu Gly Lys  
 900 905 910



Glu Glu Ala Phe Phe Ser Gly Gln  
915 920

<210> 53  
<211> 642  
<212> DNA  
<213> *Methylobacter 16a*

<220>  
<223> ORF8

<220>  
<223> norC gene

<400> 53  
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cgctgggcgt cggaacatt ctggaaaaaa accgcgatct gggtcaccgg cggatcgttc 120  
gtgttgctgg tgatcttgac cttcgactcg ctggcgaaga tttccgctgg cggccccagg 180  
gtgccggcct tcgacgtcat caacaaagac gtcagttacc gtttcgacaa ggaaaaacaa 240  
cgctaccaac cagtgatcgg cgacgacgcc cctctgtttg gcaaaaccct gagcgaggaa 300  
gaagccgaaa aactggtcga cctgggcaag aaaaccgtgc aggccaagaa ctgcatgaac 360  
tgccataccc tgctcggaat tggcgcttat tatgcgcccg acttgaccaa ggcctggctg 420  
gaccagggct ggatcgccaa ggagtcgcgc gagcaaatga tggtaattt cctgctcgat 480  
cccgaagaaa atgcccgcac cttcggctcc aaccgcaaga tgccgaatct cgacatcacg 540  
caacaggagg ccgaggcat cgctgccttt ttgaaatgga tggcatccat cgacaccaat 600  
ggttttccgc ataatttcat cgcgctgggc gaagaggaca aa 642

<210> 54  
<211> 214  
<212> PRT  
<213> *Methylobacter 16a*

<220>  
<223> NorC

<400> 54  
Met ala Thr Lys Pro Asn Ile His Ile Asn Leu Glu Val Val Met Thr  
1 5 10 15  
Glu Gln Val Pro Arg Trp Ala Ser Glu Thr Phe Trp Lys Lys Thr Ala  
20 25 30  
Ile Trp Val Thr Gly Gly Ser Phe Val Leu Leu Val Ile Leu Thr Phe  
35 40 45  
Asp Ser Leu Ala Lys Ile Ser Ala Gly Gly Pro Arg Val Pro Ala Phe  
50 55 60  
Asp Val Ile Asn Lys Asp Val Ser Tyr Arg Phe Asp Lys Glu Lys Gln  
65 70 75 80  
Arg Tyr Gln Pro Val Ile Gly Asp Asp Ala Pro Leu Phe Gly Lys Thr  
85 90 95  
Leu Ser Glu Glu Glu Ala Glu Lys Leu Val Asp Leu Gly Lys Lys Thr  
100 105 110  
Val Gln Ala Lys Asn Cys Met Asn Cys His Thr Leu Leu Gly Asn Gly  
115 120 125

Ala Tyr Tyr Ala Pro Asp Leu Thr Lys Ala Trp Leu Asp Gln Gly Trp  
 130 135 140

Ile Ala Lys Glu Ser Arg Glu Gln Met Met Val Asn Phe Leu Leu Asp  
 145 150 155 160

Pro Glu Lys Asn Ala Arg Thr Phe Gly Ser Asn Arg Lys Met Pro Asn  
 165 170 175

Leu Asp Ile Thr Gln Gln Glu Ala Glu Gly Ile Val Ala Phe Leu Lys  
 180 185 190

Trp Met ala Ser Ile Asp Thr Asn Gly Phe Pro His Asn Phe Ile Ala  
 195 200 205

Leu Gly Glu Glu Asp Lys  
 210

<210> 55  
 <211> 1503  
 <212> DNA  
 <213> *Methylobionas* 16a

<220>  
 <223> ORF9

<220>  
 <223> norB gene

<400> 55  
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 cacgccgact tcatggccaa tccgcatttg accggcgccg aaaagctggc ggtgcattac 120  
 ttcacogtgc ccatgggtgct gttcatggcg caattgctgt tcggcctgct ggccggcctg 180  
 caattcatct tcccgagttt tttatacgaa atcctggatt tcaacgtcaa ccgcatgggtg 240  
 cacatcaatg ccatgggtgt gtggatgctg tacggccttt tgggctcggg gtactggttt 300  
 ctggaagacg agagcggcgt cgagatcgtc ggcttgaaat gggggcaact ggcgttttgg 360  
 gtgctgaccg gtgcggtcgc gctgatcaac gaaggcgcgc aatacatcga agccccgcgc 420  
 ggcaacgaca cttcgctgtg gctgatcaac gaaggcgcgc aatacatcga agccccgcgc 480  
 tgggcccaga tcggcatcgt cgccgtggta ttgacctct tttacaacgt cgccgccacc 540  
 ttgcgcaaaag gcaaatgggt cgccattgcc ggcgtgttga ccctggatct ggtggccctg 600  
 gccggcttgt atctggccgg catgttctac gtcaccaata ttcgggtcga ccaatactgg 660  
 tgggtggtggg tgatccatct atgggtcgaa gcgacctggg aagtgcgtgt tggctgcata 720  
 atggcctgga gcctgatgaa gctgctgggc gtgcgcgcgc aggtcgtaca aacttggttg 780  
 tacatcgaag ttgctttgat gttcggctcc ggcattcttg gcctgggtca tcaactattc 840  
 tggatcgcca cgccggaata ctggttcagc atcggcggct tcttctcggc gctggaaccg 900  
 attccgctgg tagcaatggt cgtgcattcc atttacgatt ccggcgtgca caagtttaaa 960  
 aacagcaatc accccgccct ggcctggatc atcgcccata ctctcggaac ctctctgggc 1020  
 gccggcgttt ggggattcat gcacacgctg ccgcaaatca acctgtacac ccacggcacg 1080  
 caatggtcgg cctcgacacg ccacctggcc ttcttcggcg cctatgacac catcaacatc 1140  
 gccttcttct acctggcggc gcagcaggcg cgcggcaacg tctggatggg cgggtgactg 1200  
 atcaacggct ggcgctggaa agcggcggcg attttgctaa atctgggcgt gttgggcatg 1260  
 accgtggcgc tattgatcgc cggttacgag caatcgttta tcgaacgcgc cgtcgaaggc 1320  
 tcgacctggg ccggttactt cgccgcgcaa aaccaccgt ggttcattga agccatggtc 1380  
 tggcgcatgg tattcggtt gatgacggc gtcggcggcg gcctgtgtgt ctgggacttg 1440  
 ctggaatcgc gcaaaaggcga acagcggccc gcggcggtga ttgccggttg aacggttgcg 1500  
 gaa 1503

<210> 56  
 <211> 501

&lt;212&gt; PRT

<213> *Methylobacterium* 16a

&lt;220&gt;

&lt;223&gt; NorB

&lt;400&gt; 56

Met Thr Leu Gln Ala Tyr Gln Glu Lys Ala Ala Val Cys Trp Ala Gly  
 1 5 10 15

Cys Lys Gln Arg His Ala Asp Phe Met ala Asn Pro His Leu Thr Gly  
 20 25 30

Gly Gln Lys Leu Ala Val His Tyr Phe Thr Val Ala Met Val Leu Phe  
 35 40 45

Met ala Gln Leu Leu Phe Gly Leu Leu Ala Gly Leu Gln Phe Ile Phe  
 50 55 60

Pro Ser Phe Leu Tyr Glu Ile Leu Asp Phe Asn Val Asn Arg Met Val  
 65 70 75 80

His Ile Asn Ala Met Val Val Trp Met Leu Tyr Gly Phe Leu Gly Ser  
 85 90 95

Val Tyr Trp Phe Leu Glu Asp Glu Ser Gly Val Glu Ile Val Gly Leu  
 100 105 110

Lys Trp Gly Gln Leu Ala Phe Trp Val Leu Thr Gly Ala Val Ala Leu  
 115 120 125

Val Val Leu Val Tyr Leu Phe Ile Gln Ile Gly Ala Gly Asn Asp Thr  
 130 135 140

Ser Leu Trp Leu Ile Asn Glu Gly Arg Glu Tyr Ile Glu Ala Pro Arg  
 145 150 155 160

Trp Ala Asp Ile Gly Ile Val Ala Val Val Leu Thr Phe Phe Tyr Asn  
 165 170 175

Val Ala Ala Thr Phe Ala Lys Gly Lys Trp Ser Gly Ile Ala Gly Val  
 180 185 190

Leu Thr Leu Asp Leu Val Ala Leu Ala Gly Leu Tyr Leu Ala Gly Met  
 195 200 205

Phe Tyr Val Thr Asn Ile Ser Val Asp Gln Tyr Trp Trp Trp Trp Val  
 210 215 220

Ile His Leu Trp Val Glu Ala Thr Trp Glu Val Leu Val Gly Cys Ile  
 225 230 235 240

Met ala Trp Ser Leu Met Lys Leu Leu Gly Val Arg Arg Gln Val Val  
 245 250 255

Gln Thr Trp Leu Tyr Ile Glu Val Ala Leu Met Phe Gly Ser Gly Ile  
 260 265 270

Leu Gly Leu Gly His His Tyr Phe Trp Ile Gly Thr Pro Glu Tyr Trp  
 275 280 285

Phe Ser Ile Gly Gly Phe Phe Ser Ala Leu Glu Pro Ile Pro Leu Val  
 290 295 300  
 Ala Met Val Val His Ser Ile Tyr Asp Ser Gly Val His Lys Phe Lys  
 305 310 315 320  
 Asn Ser Asn His Pro Ala Leu Ala Trp Ile Ile Ala His Thr Phe Gly  
 325 330 335  
 Asn Phe Leu Gly Ala Gly Val Trp Gly Phe Met His Thr Leu Pro Gln  
 340 345 350  
 Ile Asn Leu Tyr Thr His Gly Thr Gln Trp Ser Ala Ser His Gly His  
 355 360 365  
 Leu Ala Phe Phe Gly Ala Tyr Ala Thr Ile Asn Ile Ala Phe Phe Tyr  
 370 375 380  
 Leu Ala Ala Gln Gln Ala Arg Gly Asn Val Trp Met Gly Gly Asp Leu  
 385 390 395 400  
 Ile Asn Gly Trp Arg Trp Lys Ala Ala Ala Ile Leu Leu Asn Leu Gly  
 405 410 415  
 Val Leu Gly Met Thr Val Ala Leu Leu Ile Ala Gly Tyr Glu Gln Ser  
 420 425 430  
 Phe Ile Glu Arg Ala Val Glu Gly Ser Thr Trp Ala Gly Tyr Phe Ala  
 435 440 445  
 Ala Gln Asn His Pro Trp Phe Met Gln Ala Met Val Trp Arg Met Val  
 450 455 460  
 Phe Gly Leu Met Thr Ala Val Gly Gly Gly Leu Leu Phe Trp Asp Leu  
 465 470 475 480  
 Leu Glu Ile Gly Lys Gly Glu Gln Arg Pro Ala Ala Val Ile Ala Gly  
 485 490 495  
 Gly Thr Val Ala Glu  
 500

<210> 57  
 <211> 2253  
 <212> DNA  
 <213> *Methylobionas* 16a

<220>  
 <223> ORF10

<220>  
 <223> norZ gene

<400> 57  
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 ctgggctttg caggcctgat cgtcatcaca tcgctggcct atcgtaatgc tccgcogatt 120  
 ccggcccaaa tcgttgatgc acaagggtgt cgcctgtttt ccgggtgacga aatcaaagaa 180  
 ggccaggcta tctttctcaa atacgggttg atgaacaacg gcagtatctg gggatcatggc 240  
 gcataacttg ggccagatta ttcggccgag gcattgcacc gaatcggcga gaacaccgcc 300

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actatcattg cccagcagca ataccaacag ccactttcct cactcacgcc cggccaattg 360
gcccgcgtgt atgcacaaac cgcagtcgag ctaaagacca atcattatga tggcgccagc 420
gcaacgttgc gtctgaccgt gccggagaca tccgcctatc gtaagcagat cgcttattgg 480
acggattatt tcctgaatcc tgaacggaat ggcggactca aacgtggatt gatcagcgat 540
ccgactgaac tgcgccagtt taccgccttc atcacatgga ctgcctgggc ctcggtggcc 600
aaccgccccg gcgagaacta ctctacacc aacaattttc catacgaccc cagcgtcggg 660
aatatgcccc ttcccgtgct gctgttatgg agtgcgttga gccttatcgt gctgctggcc 720
ggtattggaa tcgtacttct gatgtttgga aaattcgatt atcttggctg gatttagcaca 780
ggacatcatg tacatcctca tctgttgctt gggcaagcca gtgcccgtca actagcactg 840
gtgaaatttt tcgtggtggt ggcgctgctg tttcttgctc agaccttggg gggcggtgca 900
acggcgcaact atcgcccgga tccaggcagt ttttacggcc ttgagctgga gaagctattt 960
cccagcaatc tgatgcgcac ctggcatcta caaacgcgg ttttctggat tgccaccgct 1020
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aaacctttag tcaggctggt cctgctagct tcttgccga ttccgctgtt ctacatcccg 1380
gcactcttct tcggcgcaaa gaccaacttc acagtggctg atacctggcg cttctggatc 1440
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ttttatcaac tgggtcttac ccagcgcaac gttgcgcttc gagtgattta cctcgacgcc 1560
atcctctatt tcgtcgccgg cctgattggt accggccatc actgggtattt taccggccag 1620
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ctgctgactc tggacgcctg ggatttcgtg cgcaccacac gcgtgactg cgacgtctgc 1740
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tatgaagtgc gaacccaact gacaccaac catggccatg ccgcgatgat gggggtattc 1920
ggcatgctgg cactggcact gatggtattc gtgttgccgc agaccagctc cgatttgccg 1980
tgggtcgaca tcgagaaata cgtaagggtc ggattttggg gctccaatgt tggcctggct 2040
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catggatact ggcctgcgcg cagccttgat tacatcgga gcgaaaggtc gcgcctgatc 2160
gaatggctac gtctgcccgg tgatctgcta tttatcctgt ttggcgccat accgttggca 2220
atcgcatcca tcaaaggctg gctggatgtg cat 2253

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&lt;210&gt; 48

&lt;211&gt; 751

&lt;212&gt; PRT

<213> *Methylobionas* 16a

&lt;220&gt;

&lt;223&gt; NorZ

&lt;400&gt; 48

```

Met Thr Lys Thr Pro Asp Leu Ser Pro Trp Trp Leu Arg Thr Val Leu
 1             5             10             15

```

```

Ile Val Met Val Leu Gly Phe Ala Gly Leu Ile Val Ile Thr Ser Leu
             20             25             30

```

```

Ala Tyr Arg Asn Ala Pro Pro Ile Pro Ala Gln Ile Val Asp Ala Gln
             35             40             45

```

```

Gly Val Arg Leu Phe Ser Gly Asp Glu Ile Lys Glu Gly Gln Ala Ile
             50             55             60

```

```

Phe Leu Lys Tyr Gly Leu Met Asn Asn Gly Ser Ile Trp Gly His Gly
             65             70             75             80

```

```

Ala Tyr Leu Gly Pro Asp Tyr Ser Ala Glu Ala Leu His Arg Ile Gly
             85             90             95

```

Glu Asn Thr Ala Thr Ile Ile Ala Gln Gln Gln Tyr Gln Gln Pro Leu  
 100 105 110  
 Ser Ser Leu Thr Pro Gly Gln Leu Ala Ala Val Tyr Ala Gln Thr Ala  
 115 120 125  
 Val Glu Leu Lys Thr Asn His Tyr Asp Ala Ala Ser Ala Thr Leu Arg  
 130 135 140  
 Leu Thr Val Pro Glu Thr Ser Ala Tyr Arg Lys Gln Ile Ala Tyr Trp  
 145 150 155 160  
 Thr Asp Tyr Phe Leu Asn Pro Glu Arg Asn Gly Gly Leu Lys Arg Gly  
 165 170 175  
 Leu Ile Ser Asp Pro Thr Glu Leu Arg Gln Phe Thr Ala Phe Ile Thr  
 180 185 190  
 Trp Thr Ala Trp Ala Ser Val Ala Asn Arg Pro Gly Glu Asn Tyr Ser  
 195 200 205  
 Tyr Thr Asn Asn Phe Pro Tyr Asp Pro Ser Val Gly Asn Met Pro Val  
 210 215 220  
 Pro Gly Ala Leu Leu Trp Ser Ala Leu Ser Leu Ile Val Leu Leu Ala  
 225 230 235 240  
 Gly Ile Gly Ile Val Leu Leu Met Phe Gly Lys Phe Asp Tyr Leu Gly  
 245 250 255  
 Trp Ile Ser Thr Gly His His Val His Pro His Leu Leu Pro Gly Gln  
 260 265 270  
 Ala Ser Ala Gly Gln Leu Ala Leu Val Lys Phe Phe Val Val Val Ala  
 275 280 285  
 Leu Leu Phe Leu Ala Gln Thr Leu Val Gly Gly Ala Thr Ala His Tyr  
 290 295 300  
 Arg Ala Asp Pro Gly Ser Phe Tyr Gly Leu Glu Leu Glu Lys Leu Phe  
 305 310 315 320  
 Pro Ser Asn Leu Met Arg Thr Trp His Leu Gln Thr Ala Val Phe Trp  
 325 330 335  
 Ile Ala Thr Ala Phe Val Ala Ala Ala Leu Phe Leu Gly Arg Ser Leu  
 340 345 350  
 Arg Asn Asp Glu Pro Arg Trp Phe Ala Gly Trp Val His Leu Leu Phe  
 355 360 365  
 Gly Ala Phe Ala Val Val Ile Gly Gly Ser Leu Leu Gly Glu Trp Ala  
 370 375 380  
 Gly Ile Ser Gln Met Leu Asp Gln Trp Trp Phe Trp Leu Gly Asn Gln  
 385 390 395 400  
 Gly Trp Glu Tyr Leu Glu Leu Gly Arg Leu Trp Gln Tyr Leu Leu Ile  
 405 410 415

Ala Gly Leu Leu Ala Trp Phe Thr Leu Leu Phe Lys Leu Leu Gln Pro  
 420 425 430  
 Asp Thr Leu Asn Asp Ser Glu Ala Lys Pro Leu Val Arg Leu Phe Leu  
 435 440 445  
 Leu Ala Ser Leu Ala Ile Pro Leu Phe Tyr Ile Pro Ala Leu Phe Phe  
 450 455 460  
 Gly Ala Lys Thr Asn Phe Thr Val Val Asp Thr Trp Arg Phe Trp Ile  
 465 470 475 480  
 Ile His Leu Trp Val Glu Gly Phe Phe Glu Phe Phe Ala Thr Thr Leu  
 485 490 495  
 Val Ala Leu Leu Phe Tyr Gln Leu Gly Leu Thr Gln Arg Asn Val Ala  
 500 505 510  
 Leu Arg Val Ile Tyr Leu Asp Ala Ile Leu Tyr Phe Val Gly Gly Leu  
 515 520 525  
 Ile Gly Thr Gly His His Trp Tyr Phe Thr Gly Gln Ser Ser Val Asn  
 530 535 540  
 Met ala Leu Ser Ala Met Val Ser Val Leu Glu Val Val Pro Leu Thr  
 545 550 555 560  
 Leu Leu Thr Leu Asp Ala Trp Asp Phe Val Arg Thr Thr Arg Ala Asp  
 565 570 575  
 Cys Asp Val Cys Gly Lys Pro Val Ala Ile Pro His Lys Trp Thr Phe  
 580 585 590  
 Tyr Phe Leu Met ala Val Gly Phe Trp Asn Phe Val Gly Ala Gly Ile  
 595 600 605  
 Phe Gly Phe Leu Ile Asn Leu Pro Ile Val Ser Tyr Tyr Glu Val Gly  
 610 615 620  
 Thr Gln Leu Thr Pro Asn His Gly His Ala Ala Met Met Gly Val Phe  
 625 630 635 640  
 Gly Met Leu Ala Leu Ala Leu Met Val Phe Val Leu Arg Gln Thr Ser  
 645 650 655  
 Ser Asp Leu Arg Trp Val Asp Ile Glu Lys Tyr Val Arg Val Gly Phe  
 660 665 670  
 Trp Gly Ser Asn Val Gly Leu Ala Leu Met Leu Ile Met Ser Leu Phe  
 675 680 685  
 Pro Ser Gly Val Leu Gln Val Trp Asp Val Val Gln His Gly Tyr Trp  
 690 695 700  
 His Ala Arg Ser Leu Asp Tyr Ile Gly Ser Glu Arg Ser Arg Leu Ile  
 705 710 715 720  
 Glu Trp Leu Arg Leu Pro Gly Asp Leu Val Phe Ile Leu Phe Gly Ala  
 725 730 735

Ile Pro Leu Ala Ile Ala Ser Ile Lys Gly Trp Leu Asp Val His  
 740 745 750

<210> 59  
 <211> 1581  
 <212> DNA  
 <213> *Methylobacterium* 16a

<220>  
 <223> ORF11

<220>  
 <223> norS gene

<400> 59  
 atgatgaaaa caacaactaa aagacgactg aatcaatccc ttctggcgag tgctatcgcc 60  
 gcgttactgt cgtccggtgc ggtgctggcg aaatccgaca gccacacga catctacatg 120  
 gataattgcg ccagctgccg cggcgcgatg caggtgggtg atctggcgcc agccttgaat 180  
 gccgatacct tgaaaggtcg tagccctacg gcgttgcgta ccatcgatcat ggccggcagc 240  
 ttcgatacgc tgatgcctcc cttctacggc aaactgagcg acgacgagat tcgcggcgtg 300  
 atcaagcatt tgcaggaaac cccgaaacag ccgaatccgg cctggaccat cgacgacatg 360  
 aagaagtcct tgaaggttta cgtcaaggat gagagcaccg tgcctggcaa gccgactttc 420  
 caaatcgata acatcgataa tctgatcggc gtggcggcac gcggcaataa cggccgtggc 480  
 gaagggtcca aagctatttt catcaacagc accaaccatc aaaaagtcgg cgaagtggct 540  
 accggcaccg ccgcgcatat catcgacttc aatcctgccg acccgcgctg ggcttacgta 600  
 aaaaccgaca ccgccgagat tttcaaggta gatttgtatt cgatgcaggc ggtccgcagc 660  
 atcaagacag gttacaacgg ccccgcatg ggggtatccc gcgacggcaa atacatcatg 720  
 gccggctcct tcgtgccgca taacgccgta atcctggatg ccgaaaccct ggaaccgttg 780  
 aaaaacctcg aactggaagg catcgatccc gacggtaaac atgtttcttc cgactcgggc 840  
 atgatcatcg gtaccctta tgcgacgtg ttccgcatg cgctggaaaa tgcggccag 900  
 gtctggatcg tcgattacaa caaagaaggc ttcccggtca ccaaatcga gaaagtgggc 960  
 cgtcacttgc acgacgcctt cctgacgcat ggcggaaga aactgatggt ggcttcttat 1020  
 gacgacagca tcgtcgccgc gatcgatctg gaaaaacgcg aactgatcaa gcaattgccg 1080  
 gcgggttggtg tgccgcacgt cgggtggcgc gcggcggtcg tgggtgatgg tcgtaccttg 1140  
 ggcttcggta ccaactttgg cgattcgac aagatggtcg tcagcgtttg ggatttgac 1200  
 aaaaaggaa tcgtcaaaac agtaccggtt tcaggtggca ctgaatcgcc tgcggctcat 1260  
 gccaacgcac cttatgtgcg ggttgacatc atcagcaaag acagacgtgc acgcaccatt 1320  
 cagttgatcg acaagaaaac cctggaagtt gccaaaactc tggatgtcgg cggccacgcc 1380  
 tacttccggg aatatagcgc cgacggcaaa ttctctatg tcagtgcgg ctacaatggc 1440  
 gacgaagtgc tggtttacga ttccaatacc ttgcaaaaag tggcgaccgt gccgatggaa 1500  
 agtctgctg gtatctctc cagaggccgt gtcaaatata tgactcgcg tctgtcacct 1560  
 gacgaatg agcaaggcaa a 1581

<210> 60  
 <211> 527  
 <212> PRT  
 <213> *Methylobacterium* 16a

<220>  
 <223> NorS

<400> 60  
 Met Met Lys Thr Thr Thr Lys Arg Arg Leu Asn Gln Ser Leu Leu Ala  
 1 5 10 15  
 Ser Ala Ile Ala Ala Leu Leu Ser Ser Gly Ala Val Leu Ala Lys Ser  
 20 25 30



Asp Ser Pro His Asp Ile Tyr Met Asp Asn Cys Ala Ser Cys His Gly  
 35 40 45  
 Ala Asp His Gly Gly Tyr Leu Ala Pro Ala Leu Asn Ala Asp Thr Leu  
 50 55 60  
 Lys Gly Arg Ser Pro Thr Ala Leu Arg Thr Ile Val Met ala Gly Ser  
 65 70 75 80  
 Phe Asp Thr Leu Met Pro Pro Phe Tyr Gly Lys Leu Ser Asp Asp Glu  
 85 90 95  
 Ile Arg Gly Val Ile Lys His Leu Gln Glu Thr Pro Lys Gln Pro Asn  
 100 105 110  
 Pro Ala Trp Thr Ile Asp Asp Met Lys Lys Ser Leu Lys Val Tyr Val  
 115 120 125  
 Lys Asp Glu Ser Thr Leu Pro Gly Lys Pro Thr Phe Gln Ile Asp Asn  
 130 135 140  
 Ile Asp Asn Leu Ile Gly Val Ala Ala Arg Gly Lys Tyr Gly Arg Gly  
 145 150 155 160  
 Glu Gly Ser Lys Ala Ile Phe Ile Asn Ser Thr Asn His Gln Lys Val  
 165 170 175  
 Gly Glu Val Ala Thr Gly Thr Ala Ala His Ile Ile Asp Phe Asn Pro  
 180 185 190  
 Ala Asn Pro Arg Trp Ala Tyr Val Lys Thr Asp Thr Ala Glu Ile Phe  
 195 200 205  
 Lys Val Asp Leu Tyr Ser Met Gln Ala Val Arg Ser Ile Lys Thr Gly  
 210 215 220  
 Tyr Asn Gly Pro Gly Met Gly Val Ser Arg Asp Gly Lys Tyr Ile Met  
 225 230 235 240  
 Ala Gly Ser Phe Val Pro His Asn Ala Val Ile Leu Asp Ala Glu Thr  
 245 250 255  
 Leu Glu Pro Leu Lys Thr Phe Glu Leu Glu Gly Ile Asp Pro Asp Gly  
 260 265 270  
 Lys His Val Ser Ser Asp Ser Gly Met Ile Ile Gly Thr Pro Tyr Ala  
 275 280 285  
 Asp Val Phe Ala Ile Ala Leu Glu Asn Ala Gly Gln Val Trp Ile Val  
 290 295 300  
 Asp Tyr Asn Lys Glu Gly Phe Pro Val Thr Lys Ile Glu Lys Val Gly  
 305 310 315 320  
 Arg His Leu His Asp Ala Phe Leu Thr His Gly Gly Lys Lys Leu Met  
 325 330 335  
 Val Ala Ser Tyr Asp Asp Ser Ile Val Ala Ala Ile Asp Leu Glu Lys  
 340 345 350

Arg Glu Leu Ile Lys Gln Leu Pro Ala Gly Cys Val Pro His Val Gly  
 355 360 365  
 Gly Gly Ala Ala Val Val Val Asp Gly Arg Thr Leu Gly Phe Gly Thr  
 370 375 380  
 Asn Phe Gly Asp Cys Asp Lys Met Val Val Ser Val Trp Asp Leu Asp  
 385 390 395 400  
 Lys Met Glu Val Val Lys Gln Val Pro Val Ser Gly Gly Thr Glu Ser  
 405 410 415  
 Pro Ala Ala His Ala Asn Ala Pro Tyr Val Ala Val Asp Ile Ile Ser  
 420 425 430  
 Lys Asp Arg Arg Ala Arg Thr Ile Gln Leu Ile Asp Lys Lys Thr Leu  
 435 440 445  
 Glu Val Ala Lys Thr Leu Asp Val Gly Gly His Ala Tyr Phe Pro Glu  
 450 455 460  
 Tyr Ser Ala Asp Gly Lys Phe Leu Tyr Val Ser Ala Gly Tyr Asn Gly  
 465 470 475 480  
 Asp Glu Val Val Val Tyr Asp Ser Asn Thr Leu Gln Lys Val Ala Thr  
 485 490 495  
 Val Pro Met Glu Ser Pro Ala Gly Ile Phe Ser Arg Gly Arg Val Lys  
 500 505 510  
 Tyr Met Thr Arg Gly Leu Ser Pro Asp Glu Met Glu Gln Gly Lys  
 515 520 525

**Terpenoid genes**

<210> 61  
 <211> 1860  
 <212> DNA  
 <213> *Methylomonas* 16a

<220>  
 <223> DXS

<400> 61  
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 gcgctgtoca aggaccagct ccagcaactg gctgacgagg tgcgcgggcta tctgacccac 120  
 acggtcagca tttccggcgg ccattttgcg gccggcctcg gcaccgtgga actgaccgtg 180  
 gccttgcaat atgtgttcaa taccctcgct gatcagttgg tctgggacgt gggccatcag 240  
 gcctatccgc acaagattct gaccggctcg aaggagcgca tgccgacat tcgcaccctg 300  
 ggccgggtgt cagcctttcc ggccggggac gagagcgaat acgatgcctt cggcgtcggc 360  
 cattccagca cctcgatcag cgccggcactg ggcattggcca ttgcgtcgca gctgcgcggc 420  
 gaagacaaga agatggtagc catcatcggc gacggttcca tcaccggcgg catggcctat 480  
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 agcaagtttt attcgtcggc gcgggaagag agcaagaaag ctctggccaa gatgccgtcg 660  
 gtgtgggaac tggcgcgcaa gaccgaggaa cacgtgaagg gcatgatcgt gcccggtacc 720  
 ttgttcgagg aattgggctt caattatttc ggcccgatcg acggccatga tgtcgagatg 780  
 ctggtgtcga ccctggaaaa tctgaaggat ttgaccgggc cgggtattcct gcatgtggtg 840  
 accaagaagg gcaaaggcta tgcgccagcc gagaaagacc cggtggccta ccatggcgtg 900

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ccggctttcg atccgaccaa ggatttcctg cccaaggcgg cgccgtcgcc gcatccgacc 960
tataccgagg tggtcggccg ctggctgtgc gacatggcgg ctcaagacga gcgcttgctg 1020
ggcatcacgc cggcgatgcg cgaaggctct ggtttggtgg aattctcaca gaaatttccg 1080
aatcgctatt tcgatgtcgc catcgccgag cagcatgcgg tgaccttggc cgccggccag 1140
gcctgccagg ggcceaagcc ggtggtggcg atttattcca ccttcctgca acgcggttac 1200
gatcagttga tccacgacgt ggccttgacg aacttagata tgctctttgc actggatcgt 1260
gcgggcttgg tcggcccggg tggaccgacc catgctggcg cctttgatta cagctacatg 1320
cgctgtattc cgaacatgct gatcatggct ccagccgacg agaacgagtg caggcagatg 1380
ctgaccaccg gcttccaaca ccatggcccg gcttcggtgc gctatccgcg cggcaaggcg 1440
ccgggggccc caatcgatcc gaccctgacc gcgctggaga tcggcaaggc cgaagtcaga 1500
caccacggca gccgcacgc cattctggcc tggggcagca tggtcacgcc tgccgtcgaa 1560
gcgggcaagc agctggggcg gacggtggtg aacatgcgtt tcgtcaagcc gttcgatcaa 1620
gccttggtgc tgggaattggc caggacgcac gatgtgttcg tcaccgtcga ggaaaacgtc 1680
atgcgcggcg gcgctggcag tgcgatcaac accttcctgc aggcgcagaa ggtgctgatg 1740
ccggtctgca acatcggcct gcccgaccgc ttcgtcgagc aaggtagtcg cgaggaattg 1800
ctcagcctgg tcggcctcga cagcaagggc atcctcgcca ccatcgaaca gttttgcgct 1860

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&lt;210&gt; 62

&lt;211&gt; 620

&lt;212&gt; PRT

&lt;213&gt; Methylobionas 16a

&lt;220&gt;

&lt;223&gt; Amino acid sequences encoded by DXS

&lt;400&gt; 62

```

Met Lys Leu Thr Thr Asp Tyr Pro Leu Leu Lys Asn Ile His Thr Pro
  1                      5                      10                      15

```

```

Ala Asp Ile Arg Ala Leu Ser Lys Asp Gln Leu Gln Gln Leu Ala Asp
      20                      25                      30

```

```

Glu Val Arg Gly Tyr Leu Thr His Thr Val Ser Ile Ser Gly Gly His
      35                      40                      45

```

```

Phe Ala Ala Gly Leu Gly Thr Val Glu Leu Thr Val Ala Leu His Tyr
      50                      55                      60

```

```

Val Phe Asn Thr Pro Val Asp Gln Leu Val Trp Asp Val Gly His Gln
      65                      70                      75                      80

```

```

Ala Tyr Pro His Lys Ile Leu Thr Gly Arg Lys Glu Arg Met Pro Thr
      85                      90                      95

```

```

Ile Arg Thr Leu Gly Gly Val Ser Ala Phe Pro Ala Arg Asp Glu Ser
     100                      105                      110

```

```

Glu Tyr Asp Ala Phe Gly Val Gly His Ser Ser Thr Ser Ile Ser Ala
     115                      120                      125

```

```

Ala Leu Gly Met ala Ile Ala Ser Gln Leu Arg Gly Glu Asp Lys Lys
     130                      135                      140

```

```

Met Val Ala Ile Ile Gly Asp Gly Ser Ile Thr Gly Gly Met ala Tyr
     145                      150                      155                      160

```

```

Glu Ala Met Asn His Ala Gly Asp Val Asn Ala Asn Leu Leu Val Ile
     165                      170                      175

```

Leu Asn Asp Asn Asp Met Ser Ile Ser Pro Pro Val Gly Ala Met Asn  
 180 185 190  
 Asn Tyr Leu Thr Lys Val Leu Ser Ser Lys Phe Tyr Ser Ser Val Arg  
 195 200 205  
 Glu Glu Ser Lys Lys Ala Leu Ala Lys Met Pro Ser Val Trp Glu Leu  
 210 215 220  
 Ala Arg Lys Thr Glu Glu His Val Lys Gly Met Ile Val Pro Gly Thr  
 225 230 235 240  
 Leu Phe Glu Glu Leu Gly Phe Asn Tyr Phe Gly Pro Ile Asp Gly His  
 245 250 255  
 Asp Val Glu Met Leu Val Ser Thr Leu Glu Asn Leu Lys Asp Leu Thr  
 260 265 270  
 Gly Pro Val Phe Leu His Val Val Thr Lys Lys Gly Lys Gly Tyr Ala  
 275 280 285  
 Pro Ala Glu Lys Asp Pro Leu Ala Tyr His Gly Val Pro Ala Phe Asp  
 290 295 300  
 Pro Thr Lys Asp Phe Leu Pro Lys Ala Ala Pro Ser Pro His Pro Thr  
 305 310 315 320  
 Tyr Thr Glu Val Phe Gly Arg Trp Leu Cys Asp Met ala Ala Gln Asp  
 325 330 335  
 Glu Arg Leu Leu Gly Ile Thr Pro Ala Met Arg Glu Gly Ser Gly Leu  
 340 345 350  
 Val Glu Phe Ser Gln Lys Phe Pro Asn Arg Tyr Phe Asp Val Ala Ile  
 355 360 365  
 Ala Glu Gln His Ala Val Thr Leu Ala Ala Gly Gln Ala Cys Gln Gly  
 370 375 380  
 Ala Lys Pro Val Val Ala Ile Tyr Ser Thr Phe Leu Gln Arg Gly Tyr  
 385 390 395 400  
 Asp Gln Leu Ile His Asp Val Ala Leu Gln Asn Leu Asp Met Leu Phe  
 405 410 415  
 Ala Leu Asp Arg Ala Gly Leu Val Gly Pro Asp Gly Pro Thr His Ala  
 420 425 430  
 Gly Ala Phe Asp Tyr Ser Tyr Met Arg Cys Ile Pro Asn Met Leu Ile  
 435 440 445  
 Met ala Pro Ala Asp Glu Asn Glu Cys Arg Gln Met Leu Thr Thr Gly  
 450 455 460  
 Phe Gln His His Gly Pro Ala Ser Val Arg Tyr Pro Arg Gly Lys Gly  
 465 470 475 480  
 Pro Gly Ala Ala Ile Asp Pro Thr Leu Thr Ala Leu Glu Ile Gly Lys  
 485 490 495

Ala Glu Val Arg His His Gly Ser Arg Ile Ala Ile Leu Ala Trp Gly  
500 505 510

Ser Met Val Thr Pro Ala Val Glu Ala Gly Lys Gln Leu Gly Ala Thr  
515 520 525

Val Val Asn Met Arg Phe Val Lys Pro Phe Asp Gln Ala Leu Val Leu  
530 535 540

Glu Leu Ala Arg Thr His Asp Val Phe Val Thr Val Glu Glu Asn Val  
545 550 555 560

Ile Ala Gly Gly Ala Gly Ser Ala Ile Asn Thr Phe Leu Gln Ala Gln  
565 570 575

Lys Val Leu Met Pro Val Cys Asn Ile Gly Leu Pro Asp Arg Phe Val  
580 585 590

Glu Gln Gly Ser Arg Glu Glu Leu Leu Ser Leu Val Gly Leu Asp Ser  
595 600 605

Lys Gly Ile Leu Ala Thr Ile Glu Gln Phe Cys Ala  
610 615 620

&lt;210&gt; 63

&lt;211&gt; 1182

&lt;212&gt; DNA

&lt;213&gt; Methylobionas 16a

&lt;220&gt;

&lt;223&gt; DXR

&lt;400&gt; 63

```

atgaaaggta tttgcatatt gggcgctacc ggttcgatcg gtgtcagcac gctggatgtc 60
gttgccaggc atccggataa atatcaagtc gttgcgctga ccgccaacgg caatatcgac 120
gcattgtatg aacaatgcct ggcccacccat ccggagtatg ccgtgggtgt catggaaagc 180
aaggtagcag agttcaaaca ggcattgcc gcttcgccgg tagcggatat caaggtcttg 240
tcgggtagcg aggccttgca acaggtggcc acgctggaaa acgtcgatac ggtgatggcg 300
gctatcgteg gcgcggccgg attgttgccg acctggccg ccggccaaggc cggcaaaacc 360
gtgctgttgg ccaacaagga agccttggtg atgtcgggac aaatcttcat gcaggccgtc 420
agcgattccg gcgctgtgtt gctgccgata gacagcgagc acaacgccat ctttcagtgc 480
atgccggcgg gttatacgcc aggccataca gccaaacagg cgcgcgcgat tttattgacc 540
gcttccgggtg gccatttcg acggacgcgg ataagaacgt tgtccagcgt cagcccggtat 600
caggccggtg cccatccctaa atgggacatg gggcgcaaga tttcgggtcga ttccgccacc 660
atgatgaaca aaggtctcga actgatcgaa gcctgcttgt tgttcaacat ggagcccagc 720
cagattgaag tcgtcattca tccgcagagc atcattcatt cgatgggtga ctatgtcgat 780
ggttcgggtt tggcgcgagat gggtaatccc gacatgcgca cgcgatagc gcacgcgatg 840
gcctggccgg aacgctttga ctctggtgtg gcgcgcgtgg atattttcga agtagggcac 900
atggatttcg aaaaaccgga cttgaaacgg tttccttgct tgagattggc ttatgaagcc 960
atcaagtctg gtggaattat gccaacggta ttgaacgcag ccaatgaaat tgctgtcgaa 1020
gcgtttttta atgaagaagt caaattcact gacatcgcg tcatcatcga gcgcagcatg 1080
gcccagttta aaccggacga tgccggcagc ctogaattgg ttttcaggcg cgatcaagat 1140
gcgcgcgagg tggctagaga catcatcaag accttggtag ct 1182

```

&lt;210&gt; 64

&lt;211&gt; 394

&lt;212&gt; PRT

&lt;213&gt; Methylobionas 16a

&lt;220&gt;

&lt;223&gt; Amino acid sequences encoded by DXR

&lt;400&gt; 64

Met Lys Gly Ile Cys Ile Leu Gly Ala Thr Gly Ser Ile Gly Val Ser  
 1 5 10 15

Thr Leu Asp Val Val Ala Arg His Pro Asp Lys Tyr Gln Val Val Ala  
 20 25 30

Leu Thr Ala Asn Gly Asn Ile Asp Ala Leu Tyr Glu Gln Cys Leu Ala  
 35 40 45

His His Pro Glu Tyr Ala Val Val Val Met Glu Ser Lys Val Ala Glu  
 50 55 60

Phe Lys Gln Arg Ile Ala Ala Ser Pro Val Ala Asp Ile Lys Val Leu  
 65 70 75 80

Ser Gly Ser Glu Ala Leu Gln Gln Val Ala Thr Leu Glu Asn Val Asp  
 85 90 95

Thr Val Met ala Ala Ile Val Gly Ala Ala Gly Leu Leu Pro Thr Leu  
 100 105 110

Ala Ala Ala Lys Ala Gly Lys Thr Val Leu Leu Ala Asn Lys Glu Ala  
 115 120 125

Leu Val Met Ser Gly Gln Ile Phe Met Gln Ala Val Ser Asp Ser Gly  
 130 135 140

Ala Val Leu Leu Pro Ile Asp Ser Glu His Asn Ala Ile Phe Gln Cys  
 145 150 155 160

Met Pro Ala Gly Tyr Thr Pro Gly His Thr Ala Lys Gln Ala Arg Arg  
 165 170 175

Ile Leu Leu Thr Ala Ser Gly Gly Pro Phe Arg Arg Thr Pro Ile Glu  
 180 185 190

Thr Leu Ser Ser Val Thr Pro Asp Gln Ala Val Ala His Pro Lys Trp  
 195 200 205

Asp Met Gly Arg Lys Ile Ser Val Asp Ser Ala Thr Met Met Asn Lys  
 210 215 220

Gly Leu Glu Leu Ile Glu Ala Cys Leu Leu Phe Asn Met Glu Pro Asp  
 225 230 235 240

Gln Ile Glu Val Val Ile His Pro Gln Ser Ile Ile His Ser Met Val  
 245 250 255

Asp Tyr Val Asp Gly Ser Val Leu Ala Gln Met Gly Asn Pro Asp Met  
 260 265 270

Arg Thr Pro Ile Ala His Ala Met ala Trp Pro Glu Arg Phe Asp Ser  
 275 280 285

Gly Val Ala Pro Leu Asp Ile Phe Glu Val Gly His Met Asp Phe Glu  
 290 295 300

Lys Pro Asp Leu Lys Arg Phe Pro Cys Leu Arg Leu Ala Tyr Glu Ala  
 305 310 315 320  
 Ile Lys Ser Gly Gly Ile Met Pro Thr Val Leu Asn Ala Ala Asn Glu  
 325 330 335  
 Ile Ala Val Glu Ala Phe Leu Asn Glu Glu Val Lys Phe Thr Asp Ile  
 340 345 350  
 Ala Val Ile Ile Glu Arg Ser Met ala Gln Phe Lys Pro Asp Asp Ala  
 355 360 365  
 Gly Ser Leu Glu Leu Val Leu Gln Ala Asp Gln Asp Ala Arg Glu Val  
 370 375 380  
 Ala Arg Asp Ile Ile Lys Thr Leu Val Ala  
 385 390

<210> 65  
 <211> 471  
 <212> DNA  
 <213> Methylomonas 16a

<220>  
 <223> ISPF

<400> 65  
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 ttgggcggcg tcaaaatccc ttatgaaaaa ggccctggaag cccattccga cggcgacgtg 120  
 gtgctgcacg cattggcoga cgccatcttg ggagccgcga ctttgggcga catcggaaca 180  
 catttcccgg acaccgaccc caatttcaag ggcgccgaca gcaggggtgct actgcgccac 240  
 gtgtacggca tcgtcaagga aaaaggctat aaactgggtca acgccgacgt gaccatcatc 300  
 gctcagggcg cgaagatgct gccacacgtg cccggcatgc gcgccaacat tgccgccgat 360  
 ctggaaaccg atgtcgattt cattaatgta aaagccacga cgaccgagaa actgggcttt 420  
 gaggggccgta aggaaggcat cgccgtgcag gctgtggtgt tgatagaacg c 471

<210> 66  
 <211> 157  
 <212> PRT  
 <213> Methylomonas 16a

<220>  
 <223> Amino acid sequences encoded by ISPF  
 <400> 66

Met Ile Arg Val Gly Met Gly Tyr Asp Val His Arg Phe Asn Asp Gly  
 1 5 10 15  
 Asp His Ile Ile Leu Gly Gly Val Lys Ile Pro Tyr Glu Lys Gly Leu  
 20 25 30  
 Glu Ala His Ser Asp Gly Asp Val Val Leu His Ala Leu Ala Asp Ala  
 35 40 45  
 Ile Leu Gly Ala Ala Ala Leu Gly Asp Ile Gly Lys His Phe Pro Asp  
 50 55 60

Thr Asp Pro Asn Phe Lys Gly Ala Asp Ser Arg Val Leu Leu Arg His  
 65 70 75 80

Val Tyr Gly Ile Val Lys Glu Lys Gly Tyr Lys Leu Val Asn Ala Asp  
 85 90 95

Val Thr Ile Ile Ala Gln Ala Pro Lys Met Leu Pro His Val Pro Gly  
 100 105 110

Met Arg Ala Asn Ile Ala Ala Asp Leu Glu Thr Asp Val Asp Phe Ile  
 115 120 125

Asn Val Lys Ala Thr Thr Thr Glu Lys Leu Gly Phe Glu Gly Arg Lys  
 130 135 140

Glu Gly Ile Ala Val Gln Ala Val Val Leu Ile Glu Arg  
 145 150 155

<210> 67  
 <211> 693  
 <212> DNA  
 <213> Methylobionas 16a

<220>  
 <223> ISPD

<400> 67  
 atgaacccaa ccatccaatg ctggggccgtc gtgcccgcag ccggcggtcgg caaacgcatg 60  
 caagccgatc gcccacaaaca atatttaccg cttgcccgtta aaacgggtcat cgaacacaca 120  
 ctgactcgac tacttgagtc cgacgccttc caaaaagtgt cggtggcgat ttccggtcgaa 180  
 gacccttatt ggcctgaact gtccatagcc aaacacccc acatcatcac cgcgcctggc 240  
 ggcaaggaac gcgccgactc ggtgctgtct gcaatgaagg ctttagaaga tatagccagc 300  
 gaaaatgatt ggggtctggt acacgacgcc gcccgcccct gcttgacggg cagcgacatc 360  
 caccttcaaa tcgatacctt aaaaaatgac ccggtcggcg gcacccctggc cttgagttcg 420  
 cacgacacat tgaaacacgt ggatggtgac acgatcaccg caaccataga cagaaagcac 480  
 gtctggcgcg ccttgacgcc gcaaatgttc aaatacggca tggtgcgcga cgcgttgcaa 540  
 cgaaccgaag gcaatccggc cgtcaccgac gaagccagt cgctggaact tttgggccat 600  
 aaacccaaaa tcgtggaagg ccgcccggac aacatcaaaa tcacccgccc ggaagatttg 660  
 gccctggcac aattttatat ggagcaacaa gca 693

<210> 68  
 <211> 231  
 <212> PRT  
 <213> Methylobionas 16a

<220>  
 <223> Amino acid sequences encoded by ISPD

<400> 68  
 Met Asn Pro Thr Ile Gln Cys Trp Ala Val Val Pro Ala Ala Gly Val  
 1 5 10 15

Gly Lys Arg Met Gln Ala Asp Arg Pro Lys Gln Tyr Leu Pro Leu Ala  
 20 25 30

Gly Lys Thr Val Ile Glu His Thr Leu Thr Arg Leu Leu Glu Ser Asp  
 35 40 45



Ala Phe Gln Lys Val Ala Val Ala Ile Ser Val Glu Asp Pro Tyr Trp  
50 55 60

Pro Glu Leu Ser Ile Ala Lys His Pro Asp Ile Ile Thr Ala Pro Gly  
65 70 75 80

Gly Lys Glu Arg Ala Asp Ser Val Leu Ser Ala Leu Lys Ala Leu Glu  
85 90 95

Asp Ile Ala Ser Glu Asn Asp Trp Val Leu Val His Asp Ala Ala Arg  
100 105 110

Pro Cys Leu Thr Gly Ser Asp Ile His Leu Gln Ile Asp Thr Leu Lys  
115 120 125

Asn Asp Pro Val Gly Gly Ile Leu Ala Leu Ser Ser His Asp Thr Leu  
130 135 140

Lys His Val Asp Gly Asp Thr Ile Thr Ala Thr Ile Asp Arg Lys His  
145 150 155 160

Val Trp Arg Ala Leu Thr Pro Gln Met Phe Lys Tyr Gly Met Leu Arg  
165 170 175

Asp Ala Leu Gln Arg Thr Glu Gly Asn Pro Ala Val Thr Asp Glu Ala  
180 185 190

Ser Ala Leu Glu Leu Leu Gly His Lys Pro Lys Ile Val Glu Gly Arg  
195 200 205

Pro Asp Asn Ile Lys Ile Thr Arg Pro Glu Asp Leu Ala Leu Ala Gln  
210 215 220

Phe Tyr Met Glu Gln Gln Ala  
225 230

210> 69  
<211> 1632  
<212> DNA  
<213> Methylomonas 16a

<220>  
<223> PYRG

<400> 69  
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gcctcctccc tggcggcgat tctggaagac cgcggcctca aagtcactat cacaaaaactc 120  
gatccctaca tcaacgtcga ccccggcacc atgagcccgt ttcaacacgg cgaggtgttc 180  
gtgaccgaag acggtgccga aaccgatttg gaccttggcc attacgaacg gtttttgaaa 240  
accacgatga ccaagaaaaa caacttcacc accggtcagg ttacgagca ggtattacgc 300  
aacgagcgca aaggtgatta tcttggcgcg accgtgcaag tcattccaca tatcaccgac 360  
gaaatcaaac gccgggtgta tgaaagcgcc gaagggaag atgtggcatt gatcgaaagtc 420  
ggcggcacgg tggcgacat cgaatcgta ccgtttctgg aaaccatacg ccagatgggc 480  
gtggaactgg gtcgtgaccg cgccttggtc attcatttga cgctgggtgcc ttacatcaaa 540  
tcggccggcg aactgaaaac caagcccacc cagcattcgg tcaaagaact gcgcaccatc 600  
gggattcagc cggacatttt gatctgtcgt tcagaacaac cgatcccggc cagtgaacgc 660  
cgcaagatcg cgctatttac caatgtcgcc gaaaaggcgg tgatttccgc gatcgatgcc 720  
gacaccattt accgcattcc gctattgctg cgcgaacaag gcctggacga cctggtggtc 780  
gatcagttgc gcctggacgt accagcggcg gatttatcgg cctgggaaaa ggtcgtcgat 840

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ggcctgactc atccgaccga cgaagtcagc attgcgatcg tcggtaaata tgctgaccac 900
accgatgcct acaaatcgct gaatgaagcc ctgattcatg ccggcattca cacgcgccac 960
aaggtgcaaa tcagctacat cgactccgaa accatagaag ccgaaggcac cgccaaattg 1020
aaaaacgtcg atgcgatcct ggtgccgggt gggttcggcg aacgcggcgt ggaaggcaag 1080
atttctaccg tgcgttttgc ccgcgagaac aaaatcccgt atttgggcat ttgcttgggc 1140
atgcaatcgg cggtaatcga attcgccgcg aacgtggttg gcctggaagg cgcgcacagc 1200
accgaattcc tgccgaaatc gccacaccct gtgatcggct tgatcaccga atggatggac 1260
gaagccggcg aactggtcac acgcgacgaa gattccgacg tgggcggcac gatgcgtctg 1320
ggcgcgcaaa aatgccgcct gaaggctgat tccttggctt ttcagttgta tcaaaaagac 1380
gtcatcaccg agcgtcaccg ccaccgctac gaattcaaca atcaatattt aaaacaactg 1440
gaagcggccg gcatgaaaatt ttccggtaaa tcgctggacg gccgcctggt ggagatcatc 1500
gagctaccgg aacaccctg gttcctggcc tgccagttcc atcccgaatt cacctcgacg 1560
ccgcgtaacg gccacgccct attttcgggc ttcgtcgaag cggccgcaa acacaaaaca 1620
caaggcacag ca 1632

```

&lt;210&gt; 70

&lt;211&gt; 544

&lt;212&gt; PRT

&lt;213&gt; Methylomonas 16a

&lt;220&gt;

&lt;223&gt; Amino acid sequences encoded by ORF6 - PYRG

&lt;400&gt; 70

```

Met Thr Lys Phe Ile Phe Ile Thr Gly Gly Val Val Ser Ser Leu Gly
  1             5             10             15

```

```

Lys Gly Ile Ala Ala Ser Ser Leu Ala Ala Ile Leu Glu Asp Arg Gly
             20             25             30

```

```

Leu Lys Val Thr Ile Thr Lys Leu Asp Pro Tyr Ile Asn Val Asp Pro
             35             40             45

```

```

Gly Thr Met Ser Pro Phe Gln His Gly Glu Val Phe Val Thr Glu Asp
             50             55             60

```

```

Gly Ala Glu Thr Asp Leu Asp Leu Gly His Tyr Glu Arg Phe Leu Lys
             65             70             75             80

```

```

Thr Thr Met Thr Lys Lys Asn Asn Phe Thr Thr Gly Gln Val Tyr Glu
             85             90             95

```

```

Gln Val Leu Arg Asn Glu Arg Lys Gly Asp Tyr Leu Gly Ala Thr Val
             100            105            110

```

```

Gln Val Ile Pro His Ile Thr Asp Glu Ile Lys Arg Arg Val Tyr Glu
             115            120            125

```

```

Ser Ala Glu Gly Lys Asp Val Ala Leu Ile Glu Val Gly Gly Thr Val
             130            135            140

```

```

Gly Asp Ile Glu Ser Leu Pro Phe Leu Glu Thr Ile Arg Gln Met Gly
             145            150            155            160

```

```

Val Glu Leu Gly Arg Asp Arg Ala Leu Phe Ile His Leu Thr Leu Val
             165            170            175

```

```

Pro Tyr Ile Lys Ser Ala Gly Glu Leu Lys Thr Lys Pro Thr Gln His
             180            185            190

```

Ser Val Lys Glu Leu Arg Thr Ile Gly Ile Gln Pro Asp Ile Leu Ile  
 195 200 205  
 Cys Arg Ser Glu Gln Pro Ile Pro Ala Ser Glu Arg Arg Lys Ile Ala  
 210 215 220  
 Leu Phe Thr Asn Val Ala Glu Lys Ala Val Ile Ser Ala Ile Asp Ala  
 225 230 235 240  
 Asp Thr Ile Tyr Arg Ile Pro Leu Leu Leu Arg Glu Gln Gly Leu Asp  
 245 250 255  
 Asp Leu Val Val Asp Gln Leu Arg Leu Asp Val Pro Ala Ala Asp Leu  
 260 265 270  
 Ser Ala Trp Glu Lys Val Val Asp Gly Leu Thr His Pro Thr Asp Glu  
 275 280 285  
 Val Ser Ile Ala Ile Val Gly Lys Tyr Val Asp His Thr Asp Ala Tyr  
 290 295 300  
 Lys Ser Leu Asn Glu Ala Leu Ile His Ala Gly Ile His Thr Arg His  
 305 310 315 320  
 Lys Val Gln Ile Ser Tyr Ile Asp Ser Glu Thr Ile Glu Ala Glu Gly  
 325 330 335  
 Thr Ala Lys Leu Lys Asn Val Asp Ala Ile Leu Val Pro Gly Gly Phe  
 340 345 350  
 Gly Glu Arg Gly Val Glu Gly Lys Ile Ser Thr Val Arg Phe Ala Arg  
 355 360 365  
 Glu Asn Lys Ile Pro Tyr Leu Gly Ile Cys Leu Gly Met Gln Ser Ala  
 370 375 380  
 Val Ile Glu Phe Ala Arg Asn Val Val Gly Leu Glu Gly Ala His Ser  
 385 390 395 400  
 Thr Glu Phe Leu Pro Lys Ser Pro His Pro Val Ile Gly Leu Ile Thr  
 405 410 415  
 Glu Trp Met Asp Glu Ala Gly Glu Leu Val Thr Arg Asp Glu Asp Ser  
 420 425 430  
 Asp Leu Gly Gly Thr Met Arg Leu Gly Ala Gln Lys Cys Arg Leu Lys  
 435 440 445  
 Ala Asp Ser Leu Ala Phe Gln Leu Tyr Gln Lys Asp Val Ile Thr Glu  
 450 455 460  
 Arg His Arg His Arg Tyr Glu Phe Asn Asn Gln Tyr Leu Lys Gln Leu  
 465 470 475 480  
 Glu Ala Ala Gly Met Lys Phe Ser Gly Lys Ser Leu Asp Gly Arg Leu  
 485 490 495  
 Val Glu Ile Ile Glu Leu Pro Glu His Pro Trp Phe Leu Ala Cys Gln  
 500 505 510

Phe His Pro Glu Phe Thr Ser Thr Pro Arg Asn Gly His Ala Leu Phe  
 515 520 525

Ser Gly Phe Val Glu Ala Ala Lys His Lys Thr Gln Gly Thr Ala  
 530 535 540

<210> 71  
 <211> 891  
 <212> DNA  
 <213> Methylobionas 16a

<220>  
 <223> ORF7 ISPa  
 <400> 71

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gccgctctgc ctgccgaaaa catactgcca caaaccttgc atcaggccat gcgctattcc 120
gtattgaacg gcggcaaacg caccggcccc ttgttgactt atgcgaccgg tcaggctttg 180
ggcttgccgg aaaacgtgct ggatgcgcgg gcttgcgcgg tagaattcat ccatgtgtat 240
tcgctgattc acgacgatct gccggccatg gacaacgatg atctgcgcgg cggcaaacgg 300
acctgtcaca aggtttaaga cgaggccacc gccatttttg ccggcgacgc actgcaggcg 360
ctggcctttg aagttctggc caacgacccc ggcatcaccg tcgatgcccc ggctcgcttg 420
aaaatgatca cggctttgac ccgcgccagc ggctctcaag gcattggtgg cggtaagcc 480
atcgatctcg gctccgtcgg ccgcaaatg acgtgccgg aactcgaaaa catgcatatc 540
cacaagactg gcgccctgat ccgcgccagc gtcaatctgg cggcattatc caaacccgat 600
ctggatactt gcgtcgccaa gaaactggat cactatgcca aatgcatagg cttgtcgttc 660
cagggtcaaag acgacattct cgacatcgaa gccgacaccg cgacactcgg caagactcag 720
ggcaaggaca tcgataacga caaacggacc taccctgcgc tattgggcat ggctggcgcc 780
aaacaaaaag cccaggaatt gcacgaacaa gcagtcgaaa gcttaacggg atttggcagc 840
gaagccgacc tgctgcgcga actatcgctt tacatcatcg agcgcacgca c 891
```

<210> 72  
 <211> 297  
 <212> PRT  
 <213> Methylobionas 16a

<220>  
 <223> Amino acid sequences encoded by ORF7 - ISPa  
 <400> 72

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Met Ser Lys Leu Lys Ala Tyr Leu Thr Val Cys Gln Glu Arg Val Glu
  1 5 10 15

Arg Ala Leu Asp Ala Arg Leu Pro Ala Glu Asn Ile Leu Pro Gln Thr
  20 25 30

Leu His Gln Ala Met Arg Tyr Ser Val Leu Asn Gly Gly Lys Arg Thr
  35 40 45

Arg Pro Leu Leu Thr Tyr Ala Thr Gly Gln Ala Leu Gly Leu Pro Glu
  50 55 60

Asn Val Leu Asp Ala Pro Ala Cys Ala Val Glu Phe Ile His Val Tyr
  65 70 75 80

Ser Leu Ile His Asp Asp Leu Pro Ala Met Asp Asn Asp Asp Leu Arg
  85 90 95

Arg Gly Lys Pro Thr Cys His Lys Ala Tyr Asp Glu Ala Thr Ala Ile
  100 105 110
```

Leu Ala Gly Asp Ala Leu Gln Ala Leu Ala Phe Glu Val Leu Ala Asn  
           115  120  125  
 Asp Pro Gly Ile Thr Val Asp Ala Pro Ala Arg Leu Lys Met Ile Thr  
           130  135  140  
 Ala Leu Thr Arg Ala Ser Gly Ser Gln Gly Met Val Gly Gly Gln Ala  
           145  150  155  160  
 Ile Asp Leu Gly Ser Val Gly Arg Lys Leu Thr Leu Pro Glu Leu Glu  
   165  170  175  
 Asn Met His Ile His Lys Thr Gly Ala Leu Ile Arg Ala Ser Val Asn  
   180  185  190  
 Leu Ala Ala Leu Ser Lys Pro Asp Leu Asp Thr Cys Val Ala Lys Lys  
           195  200  205  
 Leu Asp His Tyr Ala Lys Cys Ile Gly Leu Ser Phe Gln Val Lys Asp  
           210  215  220  
 Asp Ile Leu Asp Ile Glu Ala Asp Thr Ala Thr Leu Gly Lys Thr Gln  
           225  230  235  240  
 Gly Lys Asp Ile Asp Asn Asp Lys Pro Thr Tyr Pro Ala Leu Leu Gly  
   245  250  255  
 Met ala Gly Ala Lys Gln Lys Ala Gln Glu Leu His Glu Gln Ala Val  
   260  265  270  
 Glu Ser Leu Thr Gly Phe Gly Ser Glu Ala Asp Leu Leu Arg Glu Leu  
           275  280  285  
 Ser Leu Tyr Ile Ile Glu Arg Thr His  
           290  295

&lt;210&gt; 73

&lt;211&gt; 855

&lt;212&gt; DNA

&lt;213&gt; Methylomonas 16a

&lt;220&gt;

&lt;223&gt; ISPE

&lt;400&gt; 73

atggattatg cggctgggtg gggcgaaaga tggcctgctc cggcaaaatt gaacttaatg 60  
 ttgaggatta ccggtcgcag gccagatggc tatcatctgt tgcaaacggt gtttcaaattg 120  
 ctogatctat gcgattggtt gacgtttcat ccggttgatg atggccgcgt gacgctgcga 180  
 aatccaatct ccggcggtcc agagcaggat gacttgactg ttcgggcggc taatttggtg 240  
 aagtctcata ccggtgtgtg gcgcggagtt tgtatcgata tcgagaaaaa tctgcctatg 300  
 ggtggtggtt tgggtggtgg aagttccgat gctgctacaa ccttggtagt tctaaatcgg 360  
 ctttggggct tgggcttgtc gaagcgtgag ttgatggatt tgggcttgag gcttggtgcc 420  
 gatgtgcctg tgtttggtt tggttgttcg gcctggggcg aaggtgtgag cgaggatttg 480  
 caggcaataa cgttgccgga acaatggttt gtcatacatta aaccgattg ccatgtgaat 540  
 actggagaaa tttttctgc agaaaatttg acaaggaata gtgcagtcgt tacaatgagc 600  
 gactttcttg caggggataa tcggaatgat tgttcggaag tggtttgcaa gttatatcga 660  
 ccggtgaaag atgcaatcga tgcgttgta tgctatgcgg aagcgagatt gacggggacc 720  
 ggtgcattgtg tgttcgctca gttttgtaac aaggaagatg ctgagagtgc gttagaagga 780

ttgaaagatc ggtggctggt gttcttggct aaaggcttga atcagttctgc gctctacaag 840  
 aaattagaac aggga 855

<210> 74

<211> 285

<212> PRT

<213> Methylobionas 16a

<220>

<223> Amino acid sequences encoded by ISPE

<400> 74

Met Asp Tyr Ala Ala Gly Trp Gly Glu Arg Trp Pro Ala Pro Ala Lys  
 1 5 10 15

Leu Asn Leu Met Leu Arg Ile Thr Gly Arg Arg Pro Asp Gly Tyr His  
 20 25 30

Leu Leu Gln Thr Val Phe Gln Met Leu Asp Leu Cys Asp Trp Leu Thr  
 35 40 45

Phe His Pro Val Asp Asp Gly Arg Val Thr Leu Arg Asn Pro Ile Ser  
 50 55 60

Gly Val Pro Glu Gln Asp Asp Leu Thr Val Arg Ala Ala Asn Leu Leu  
 65 70 75 80

Lys Ser His Thr Gly Cys Val Arg Gly Val Cys Ile Asp Ile Glu Lys  
 85 90 95

Asn Leu Pro Met Gly Gly Gly Leu Gly Gly Gly Ser Ser Asp Ala Ala  
 100 105 110

Thr Thr Leu Val Val Leu Asn Arg Leu Trp Gly Leu Gly Leu Ser Lys  
 115 120 125

Arg Glu Leu Met Asp Leu Gly Leu Arg Leu Gly Ala Asp Val Pro Val  
 130 135 140

Phe Val Phe Gly Cys Ser Ala Trp Gly Glu Gly Val Ser Glu Asp Leu  
 145 150 155 160

Gln Ala Ile Thr Leu Pro Glu Gln Trp Phe Val Ile Ile Lys Pro Asp  
 165 170 175

Cys His Val Asn Thr Gly Glu Ile Phe Ser Ala Glu Asn Leu Thr Arg  
 180 185 190

Asn Ser Ala Val Val Thr Met Ser Asp Phe Leu Ala Gly Asp Asn Arg  
 195 200 205

Asn Asp Cys Ser Glu Val Val Cys Lys Leu Tyr Arg Pro Val Lys Asp  
 210 215 220

Ala Ile Asp Ala Leu Leu Cys Tyr Ala Glu Ala Arg Leu Thr Gly Thr  
 225 230 235 240

Gly Ala Cys Val Phe Ala Gln Phe Cys Asn Lys Glu Asp Ala Glu Ser  
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Ala Leu Glu Gly Leu Lys Asp Arg Trp Leu Val Phe Leu Ala Lys Gly  
 260 265 270

Leu Asn Gln Ser Ala Leu Tyr Lys Lys Leu Glu Gln Gly  
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 ggcgcgaacc gcccgatcaa catgaacggc tttaccttcg ataccgggtc gacattcttg 180  
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 <213> Methylobionas 16a

<220>  
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 Asn Gly Phe Thr Phe Asp Thr Gly Pro Thr Phe Leu Leu Met Lys Gly  
                   50                                  55                                  60  
 Val Leu Asp Glu Met Phe Glu Leu Cys Glu Arg Arg Ser Glu Asp Tyr  
                   65                                  70                                  75                                  80  
 Leu Glu Phe Leu Pro Leu Ser Pro Met Tyr Arg Leu Leu Tyr Asp Asp  
                                   85                                  90                                  95  
 Arg Asp Ile Phe Val Tyr Ser Asp Arg Glu Asn Met Arg Ala Glu Leu  
                                   100                                  105                                  110  
 Gln Arg Val Phe Asp Glu Gly Thr Asp Gly Tyr Glu Gln Phe Met Glu  
                                   115                                  120                                  125  
 Gln Glu Arg Lys Arg Phe Asn Ala Leu Tyr Pro Cys Ile Thr Arg Asp  
                                   130                                  135                                  140  
 Tyr Ser Ser Leu Lys Ser Phe Leu Ser Leu Asp Leu Ile Lys Ala Leu  
                                   145                                  150                                  155                                  160  
 Pro Trp Leu Ala Phe Pro Lys Ser Val Phe Asn Asn Leu Gly Gln Tyr  
                                   165                                  170                                  175  
 Phe Asn Gln Glu Lys Met Arg Leu Ala Phe Cys Phe Gln Ser Lys Tyr  
                                   180                                  185                                  190  
 Leu Gly Met Ser Pro Trp Glu Cys Pro Ala Leu Phe Thr Met Leu Pro  
                                   195                                  200                                  205  
 Tyr Leu Glu His Glu Tyr Gly Ile Tyr His Val Lys Gly Gly Leu Asn  
                                   210                                  215                                  220  
 Arg Ile Ala Ala Ala Met ala Gln Val Ile Ala Glu Asn Gly Gly Glu  
                                   225                                  230                                  235                                  240  
 Ile His Leu Asn Ser Glu Ile Glu Ser Leu Ile Ile Glu Asn Gly Ala  
                                   245                                  250                                  255  
 Ala Lys Gly Val Lys Leu Gln His Gly Ala Glu Leu Arg Gly Asp Glu  
                                   260                                  265                                  270  
 Val Ile Ile Asn Ala Asp Phe Ala His Ala Met Thr His Leu Val Lys  
                                   275                                  280                                  285  
 Pro Gly Val Leu Lys Lys Tyr Thr Pro Glu Asn Leu Lys Gln Arg Glu  
                                   290                                  295                                  300  
 Tyr Ser Cys Ser Thr Phe Met Leu Tyr Leu Gly Leu Asp Lys Ile Tyr  
                                   305                                  310                                  315                                  320  
 Asp Leu Pro His His Thr Ile Val Phe Ala Lys Asp Tyr Thr Thr Asn  
                                   325                                  330                                  335  
 Ile Arg Asn Ile Phe Asp Asn Lys Thr Leu Thr Asp Asp Phe Ser Phe  
                                   340                                  345                                  350



Tyr Val Gln Asn Ala Ser Ala Ser Asp Asp Ser Leu Ala Pro Ala Gly  
 355 360 365  
 Lys Ser Ala Leu Tyr Val Leu Val Pro Met Pro Asn Asn Asp Ser Gly  
 370 375 380  
 Leu Asp Trp Gln Ala His Cys Gln Asn Val Arg Glu Gln Val Leu Asp  
 385 390 395 400  
 Thr Leu Gly Ala Arg Leu Gly Leu Ser Asp Ile Arg Ala His Ile Glu  
 405 410 415  
 Cys Glu Lys Ile Ile Thr Pro Gln Thr Trp Glu Thr Asp Glu His Val  
 420 425 430  
 Tyr Lys Gly Ala Thr Phe Ser Leu Ser His Lys Phe Ser Gln Met Leu  
 435 440 445  
 Tyr Trp Arg Pro His Asn Arg Phe Glu Glu Leu Ala Asn Cys Tyr Leu  
 450 455 460  
 Val Gly Gly Gly Thr His Pro Gly Ser Gly Leu Pro Thr Ile Tyr Glu  
 465 470 475 480  
 Ser Ala Arg Ile Ser Ala Lys Leu Ile Ser Gln Lys His Arg Val Arg  
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&lt;210&gt; 77

&lt;211&gt; 1491

&lt;212&gt; DNA

&lt;213&gt; Methylomonas 16a

&lt;220&gt;

&lt;223&gt; CRTN2

&lt;400&gt; 77

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gaattatcca atctgtattt cgtcggcggc agcgtcaatc ccggcggcgg catgccgatg 1440
gtgacgctgt ccgggcaatt ggtgagggac aagattgtgg cggatttgca a 1491

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&lt;210&gt; 78

&lt;211&gt; 497

&lt;212&gt; PRT

&lt;213&gt; Methylomonas 16a

&lt;220&gt;

&lt;223&gt; Amino acid sequences encoded by CRTN2

&lt;400&gt; 78

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Met Asn Ser Asn Asp Asn Gln Arg Val Ile Val Ile Gly Ala Gly Leu
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Gly Gly Leu Ser Ala Ala Ile Ser Leu Ala Thr Ala Gly Phe Ser Val
          20             25             30

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```

Gln Leu Ile Glu Lys Asn Asp Lys Val Gly Gly Lys Leu Asn Ile Met
          35             40             45

```

```

Thr Lys Asp Gly Phe Thr Phe Asp Leu Gly Pro Ser Ile Leu Thr Met
          50             55             60

```

```

Pro His Ile Phe Glu Ala Leu Phe Thr Gly Ala Gly Lys Asn Met ala
          65             70             75             80

```

```

Asp Tyr Val Gln Ile Gln Lys Val Glu Pro His Trp Arg Asn Phe Phe
          85             90             95

```

```

Glu Asp Gly Ser Val Ile Asp Leu Cys Glu Asp Ala Glu Thr Gln Arg
          100            105            110

```

```

Arg Glu Leu Asp Lys Leu Gly Pro Gly Thr Tyr Ala Gln Phe Gln Arg
          115            120            125

```

```

Phe Leu Asp Tyr Ser Lys Asn Leu Cys Thr Glu Thr Glu Ala Gly Tyr
          130            135            140

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```

Phe Ala Lys Gly Leu Asp Gly Phe Trp Asp Leu Leu Lys Phe Tyr Gly
          145            150            155            160

```

```

Pro Leu Arg Ser Leu Leu Ser Phe Asp Val Phe Arg Ser Met Asp Gln
          165            170            175

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Gly Val Arg Arg Phe Ile Ser Asp Pro Lys Leu Val Glu Ile Leu Asn
          180            185            190

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```

Tyr Phe Ile Lys Tyr Val Gly Ser Ser Pro Tyr Asp Ala Pro Ala Leu
          195            200            205

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Met Asn Leu Leu Pro Tyr Ile Gln Tyr His Tyr Gly Leu Trp Tyr Val
          210            215            220

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Lys Gly Gly Met Tyr Gly Met ala Gln Ala Met Glu Lys Leu Ala Val
          225            230            235            240

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Glu Leu Gly Val Glu Ile Arg Leu Asp Ala Glu Val Ser Glu Ile Gln  
 245 250 255  
 Lys Gln Asp Gly Arg Ala Cys Ala Val Lys Leu Ala Asn Gly Asp Val  
 260 265 270  
 Leu Pro Ala Asp Ile Val Val Ser Asn Met Glu Val Ile Pro Ala Met  
 275 280 285  
 Glu Lys Leu Leu Arg Ser Pro Ala Ser Glu Leu Lys Lys Met Gln Arg  
 290 295 300  
 Phe Glu Pro Ser Cys Ser Gly Leu Val Leu His Leu Gly Val Asp Arg  
 305 310 315 320  
 Leu Tyr Pro Gln Leu Ala His His Asn Phe Phe Tyr Ser Asp His Pro  
 325 330 335  
 Arg Glu His Phe Asp Ala Val Phe Lys Ser His Arg Leu Ser Asp Asp  
 340 345 350  
 Pro Thr Ile Tyr Leu Val Ala Pro Cys Lys Thr Asp Pro Ala Gln Ala  
 355 360 365  
 Pro Ala Gly Cys Glu Ile Ile Lys Ile Leu Pro His Ile Pro His Leu  
 370 375 380  
 Asp Pro Asp Lys Leu Leu Thr Ala Glu Asp Tyr Ser Ala Leu Arg Glu  
 385 390 395 400  
 Arg Val Leu Val Lys Leu Glu Arg Met Gly Leu Thr Asp Leu Arg Gln  
 405 410 415  
 His Ile Val Thr Glu Glu Tyr Trp Thr Pro Leu Asp Ile Gln Ala Lys  
 420 425 430  
 Tyr Tyr Ser Asn Gln Gly Ser Ile Tyr Gly Val Val Ala Asp Arg Phe  
 435 440 445  
 Lys Asn Leu Gly Phe Lys Ala Pro Gln Arg Ser Ser Glu Leu Ser Asn  
 450 455 460  
 Leu Tyr Phe Val Gly Gly Ser Val Asn Pro Gly Gly Gly Met Pro Met  
 465 470 475 480  
 Val Thr Leu Ser Gly Gln Leu Val Arg Asp Lys Ile Val Ala Asp Leu  
 485 490 495  
 Gln  
 497

&lt;210&gt; 79

&lt;211&gt; 1455

&lt;212&gt; DNA

&lt;213&gt; METHYLOMONAS SP.

&lt;400&gt; 79

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&lt;210&gt; 80

&lt;211&gt; 485

&lt;212&gt; PRT

&lt;213&gt; METHYLOMONAS SP.

&lt;400&gt; 80

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His Leu Leu Leu Val Ala Leu Leu Arg Pro Leu Val Leu Lys Asn Arg
 20             25             30

Leu His Arg Arg Arg Cys Phe Leu Ile Glu Asp Leu Arg Lys Met Thr
 35             40             45

Ala Gly Met Thr Cys Ser Arg Leu Pro Glu Asp Leu Asn Tyr Lys Phe
 50             55             60

Ser Ser Asn Arg Gly Gly Asn Met Lys Ile Ile Lys Asp Arg Val Ala
 65             70             75             80

Lys Leu Ser Phe Val Ala Leu Leu Ile Thr Met Thr Ala Ala Met Phe
 85             90             95

Tyr Ala Pro Thr Ala Ser Ala His Gly Glu Lys Ser Gln Ala Ala Phe
100             105             110

Met Arg Met Arg Thr Ile His Trp Phe Asp Leu Asn Trp Ser Ala Asp
115             120             125

Glu Val Ala Val Asn Asp Thr Met Thr Ile Ser Gly Lys Phe His Val
130             135             140

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Phe Ala Gly Trp Pro Glu Thr Val Asp Lys Pro Glu Val Ser Phe Leu  
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 Gly Gly Gln Leu Val Pro Arg Ser Val Ser Leu Glu Leu Gly Glu Thr  
 180 185 190  
 Tyr Glu Phe Lys Val Leu Leu Lys Ala Arg Arg Pro Gly Asp Trp His  
 195 200 205  
 Val His Thr Met Met Asn Val Gln Gly Gly Gly Pro Ile Ile Gly Pro  
 210 215 220  
 Gly Lys Trp Val Thr Ile Thr Gly Lys Met Ser Asp Phe Val Asn Pro  
 225 230 235 240  
 Val Thr Thr Leu Thr Gly Gln Thr Ile Asp Leu Glu Asp Tyr Ala Leu  
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 260 265 270  
 Trp Leu Ile Phe Trp Ile Lys Arg Pro Ile Phe Val Pro Arg His Ile  
 275 280 285  
 Ala Val Ser Thr Gly Lys Ala Asp Ser Leu Ile Ser Ala Gly Asp Lys  
 290 295 300  
 Lys Val Ala Met Ile Phe Gly Val Gly Thr Leu Val Ile Val Ala Ala  
 305 310 315 320  
 Ser Met Gly Ala Thr Asn Glu Lys Tyr Pro Val Thr Thr Pro Leu Gln  
 325 330 335  
 Ala Gly Leu Leu Arg Gly Met Lys Pro Tyr Gln Met Pro Glu Ser Thr  
 340 345 350  
 Val Ser Val Lys Val Asp Asp Ala Thr Tyr Arg Val Pro Gly Arg Ala  
 355 360 365  
 Met Gln Met Thr Leu Thr Ile Thr Asn Asn Gly Asp Ser Ala Val Arg  
 370 375 380  
 Leu Gly Glu Phe Asn Thr Ala Gly Val Arg Phe Leu Asp Ser Ser Val  
 385 390 395 400  
 His Glu Asp Glu Thr Gly Tyr Pro Asp Asp Leu Leu Ala Glu Asp Gly  
 405 410 415  
 Leu Ser Val Ser Asp Asn Ser Pro Ile Ala Pro Gly Glu Thr Arg Thr  
 420 425 430  
 Val Glu Val Thr Ala Ser Asp Ala Ala Trp Glu Val Tyr Arg Leu Ala  
 435 440 445  
 Asp Leu Ile Tyr Asp Pro Asp Ser Arg Phe Ala Gly Leu Met Phe Phe  
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Ile Pro Thr Phe Ile  
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<210> 81

<211> 1429

<212> DNA

<213> METHYLOMONAS SP.

<400> 81

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International Bureau



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14 March 2002 (14.03.2002)

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(10) International Publication Number  
**WO 02/020728 A3**

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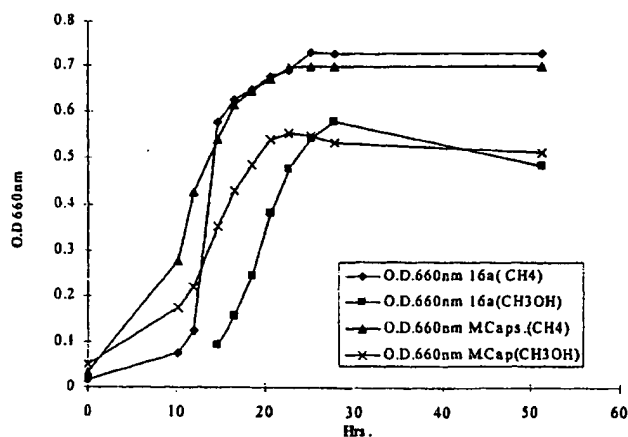
(72) Inventors; and

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[Continued on next page]

(54) Title: HIGH GROWTH METHANOTROPHIC BACTERIAL STRAIN METHYLOMONAS 16A

16a vs. MCapsulatus Growth Curve



(57) Abstract: A high growth methanotrophic bacterial strain capable of growth on a C1 carbon substrate has been isolated and characterized. The strain has the unique ability to utilize both methane and methanol as a sole carbon source and has been demonstrated to possess a functional Embden-Meyerhof carbon flux pathway. The possession of this pathway conveys an energetic advantage to the strain, making it particularly suitable as a production platform for the production of biomass from a C1 carbon source.

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**Published:**

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- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

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**(88) Date of publication of the international search report:**

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/26827

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N1/20 C12N1/30 C12N1/32 C12P1/04 C12Q1/04  
 C12Q1/68 C12R1/26 C12N1/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, MEDLINE, SEQUENCE SEARCH

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	<p>DATABASE BIOSIS 'Online!          BIOSCIENCES INFORMATION SERVICE,          PHILADELPHIA, PA, US;          Acces. No. PREV198987130157, 1988          XP002195113          abstract          &amp; SHISHKINA V N; TROTSSENKO YU A : "EFFECT          OF GLUCOSE ON THE GROWTH AND METABOLISM OF          OBLIGATE METHANOTROPHS "          MIKROBIOLOGIYA ,          vol. 57, no. 6, 1988, pages 917-923,          ---          -/--</p>	3,5

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

5 April 2002

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## INTERNATIONAL SEARCH REPORT

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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